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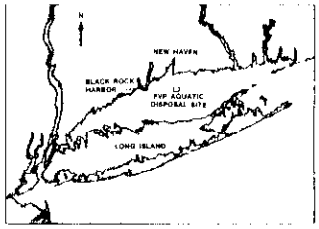

ADENYLATE ENERGY CHARGE

US Army Corps of Engineers

FIELD VERIFICATION PROGRAM (AQUATIC DISPOSAL)

TECHNICAL REPORT D-88-4

A FIELD AND LABORATORY STUDY USING ADENYLATE ENERGY CHARGE AS AN INDICATOR OF STRESS IN *MYTILUS EDULIS* AND *NEPHTYS INCISA* TREATED WITH DREDGED MATERIAL

by

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June 1988
Final Report

Approved For Public Release; Distribution Unlimited

Prepared for DEPARTMENT OF THE ARMY
US Army Corps of Engineers
Washington, DC 20314-1000

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US Environmental Protection Agency
Washington, DC 20460

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The D-series of reports includes publications of the Environmental Effects of Dredging Programs:

Dredging Operations Technical Support

Long-Term Effects of Dredging Operations

Interagency Field Verification of Methodologies for Evaluating Dredged Material Disposal Alternatives (Field Verification Program)

SUBJECT: Transmittal of Field Verification Program Technical Report Entitled "A Field and Laboratory Study Using Adenylate Energy Charge as an Indicator of Stress in *Mytilus edulis* and *Nephtys incisa* Treated with Dredged Material"

TO: All Report Recipients

1. This is one in a series of scientific reports documenting the findings of studies conducted under the Interagency Field Verification of Testing and Predictive Methodologies for Dredged Material Disposal Alternatives (referred to as the Field Verification Program or FVP). This program is a comprehensive evaluation of environmental effects of dredged material disposal under conditions of upland and aquatic disposal and wetland creation.

2. The FVP originated out of the mutual need of both the Corps of Engineers (Corps) and the Environmental Protection Agency (EPA) to continually improve the technical basis for carrying out their shared regulatory missions. The program is an expansion of studies proposed by EPA to the US Army Engineer Division, New England (NED), in support of its regulatory and dredging missions related to dredged material disposal into Long Island Sound. Discussions among the Corps' Waterways Experiment Station (WES), NED, and the EPA Environmental Research Laboratory (ERLN) in Narragansett, RI, made it clear that a dredging project at Black Rock Harbor in Bridgeport, CT, presented a unique opportunity for simultaneous evaluation of aquatic disposal, upland disposal, and wetland creation using the same dredged material. Evaluations were to be based on technology existing within the two agencies or developed during the six-year life of the program.

3. The program is generic in nature and will provide techniques and interpretive approaches applicable to evaluation of many dredging and disposal operations. Consequently, while the studies will provide detailed site-specific information on disposal of material dredged from Black Rock Harbor, they will also have great national significance for the Corps and EPA.

4. The FVP is designed to meet both Agencies' needs to document the effects of disposal under various conditions, provide verification of the predictive accuracy of evaluative techniques now in use, and provide a basis for determining the degree to which biological response is correlated with bioaccumulation of key contaminants in the species under study. The latter is an important aid in interpreting potential biological consequences of bioaccumulation. The program also meets EPA mission needs by providing an opportunity to document the application of the generic predictive hazard-assessment research strategy applicable to all wastes disposed in the aquatic environment. Therefore, the ERLN initiated exposure-assessment studies at the aquatic disposal site. The Corps-sponsored studies on environmental consequences of aquatic disposal will provide the effects assessment necessary to complement the EPA-sponsored exposure assessment, thereby allowing ERLN to develop and apply a hazard-assessment strategy. While not part of the Corps-funded FVP, the EPA exposure-assessment studies will complement the Corps' work, and together the Corps and the EPA studies will satisfy the needs of both agencies.

SUBJECT: Transmittal of Field Verification Program Technical Report Entitled "A Field and Laboratory Study Using Adenylate Energy Charge as an Indicator of Stress in *Mytilus edulis* and *Nephtys incisa* Treated with Dredged Material"

5. In recognition of the potential national significance, the Office, Chief of Engineers, approved and funded the studies in January 1982. The work is managed through the Environmental Laboratory's Environmental Effects of Dredging Programs at WES. Studies of the effects of upland disposal and wetland creation were conducted by WES, and studies of aquatic disposal were carried out by the ERLN, applying techniques worked out at the laboratory for evaluating sublethal effects of contaminants on aquatic organisms. These studies were funded by the Corps while salary, support facilities, etc., were provided by EPA. The EPA funding to support the exposure-assessment studies followed in 1983; the exposure-assessment studies are managed and conducted by ERLN.

6. The Corps and EPA are pleased at the opportunity to conduct cooperative research and believe that the value in practical implementation and improvement of environmental regulations of dredged material disposal will be considerable. The studies conducted under this program are scientific in nature and are published in the scientific literature as appropriate and in a series of Corps technical reports. The EPA will publish findings of the exposure-assessment studies in the scientific literature and in EPA report series. The FVP will provide the scientific basis upon which regulatory recommendations will be made and upon which changes in regulatory implementation, and perhaps regulations themselves, will be based. However, the documents produced by the program do not in themselves constitute regulatory guidance from either agency. Regulatory guidance will be provided under separate authority after appropriate technical and administrative assessment of the overall findings of the entire program.

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Agency

REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188
Exp Date Jun 30, 1986

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S) Technical Report D-88-4
6a. NAME OF PERFORMING ORGANIZATION See reverse 6b. OFFICE SYMBOL (*If applicable*)	7a. NAME OF MONITORING ORGANIZATION USAEWES Environmental Laboratory
6c. ADDRESS (*City, State, and ZIP Code*) Narragansett, RI 02882; Bellevue, WA 98005; Narragansett, RI 02882	7b. ADDRESS (*City, State, and ZIP Code*) PO Box 631 Vicksburg, MS 39180-0631
8a. NAME OF FUNDING/SPONSORING ORGANIZATION See reverse 8b. OFFICE SYMBOL (*If applicable*)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (*City, State, and ZIP Code*) Washington, DC 20314-1000; Washington, DC 20460	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO

11. TITLE (*Include Security Classification*)
A Field and Laboratory Study Using Adenylate Energy Charge as an Indicator of Stress in *Mytilus edulis* and *Nephtys incisa* Treated with Dredged Material

12. PERSONAL AUTHOR(S) Zaroogian, Gerald E; Rogerson, Peter F.; Hoffman, Gerald; Johnson, Mary; Johns, D. Michael; Nelson, Williams G.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (*Year, Month, Day*)	15. PAGE COUNT
Final report	FROM _____ TO _____	May 1988	166

16. SUPPLEMENTARY NOTATION
Available from National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.

17. COSATI CODES			18. SUBJECT TERMS (*Continue on reverse if necessary and identify by block number*)
FIELD	GROUP	SUB-GROUP	

19. ABSTRACT (*Continue on reverse if necessary and identify by block number*)

A study was conducted to test the applicability of adenylate energy charge (AEC) and adenine nucleotide pool concentrations as measures of biological response in the blue mussel, *Mytilus edulis*, and the marine polychaete, *Nephtys incisa*, after exposure in the laboratory and field to contaminated dredged material from Black Rock Harbor (BRH), Bridgeport, Conn. A second objective was to include field verification of laboratory results, and a third objective was to investigate residue-effect relationships between tissue concentrations of BRH contaminants and AEC and adenine nucleotide pool concentrations. This project was part of the US Environmental Protection Agency/Corps of Engineers Field Verification Program.

Biological responses were measured in a laboratory dosing system that provided constant exposure concentrations of suspended BRH sediment ranging from 0 to 10 mg/ℓ for

(Continued)

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL	22b TELEPHONE (*Include Area Code*) / 22c. OFFICE SYMBOL

6a. NAME OF PERFORMING ORGANIZATION (Continued).

US Environmental Protection Agency;
Tetra Tech;
Science Applications International Corporation

8a. NAME OF FUNDING/SPONSORING ORGANIZATION (Continued).

DEPARTMENT OF THE ARMY
US Army Corps of Engineers;
US Environmental Protection Agency

19. ABSTRACT (Continued).

M. edulis and 0 to 200 mg/ℓ for *N. incisa*. In the field, biological responses were measured in both species sampled along a transect of stations at the Central Long Island Sound disposal site. Strong exposure-residue relationships measured in laboratory experiments indicated that selected contaminants in BRH sediments were biologically available. Tissue residue concentrations, particularly of persistent compounds such as polychlorinated biphenyls, were found to be closely related to exposure concentration. This close relationship between exposure concentrations and tissue residues, as defined in laboratory experiments, was used to estimate field exposures based on tissue residues measured in field-collected *M. edulis* and *N. incisa*. The field exposures estimated from tissue residues were corroborated using estimates based on water and sediment chemistry.

The biological responses evaluated in this report included the adenine nucleotide measures of adenosine triphosphate, adenosine diphosphate, adenosine monophosphate, adenylate pool, and AEC. These responses were measured in *M. edulis* and *N. incisa* exposed to BRH sediment in the laboratory and the field. The only significant laboratory response was a reduction in adenylate pool concentration measured in *M. edulis* at BRH exposure concentrations higher than any estimated exposures in the field. The only significant field responses were station-related changes in all adenylate nucleotide concentrations measured in *N. incisa* 16 weeks postdisposal and were indicative of nonstressed organisms. This represented an exposure lasting 10 weeks longer than the longest laboratory exposure, which was 6 weeks.

The adenine nucleotide pool concentrations in organisms exposed to BRH sediments will respond in a concentration-related manner. However, these responses are relatively insensitive in *M. edulis* and are related to long exposure periods in *N. incisa*.

The apparent differences between laboratory and field responses for *M. edulis* and *N. incisa* could be explained by differences in exposures between the two situations. For *M. edulis*, the exposures were much higher in the laboratory. For *N. incisa*, the exposures in the laboratory and the field were comparable, but the field response was significant at 16 weeks postdisposal. This length of exposure far exceeded the length of any laboratory experiments.

Adenine nucleotides and AEC are important in energy transformation and in regulation of metabolic processes. Therefore, it is not surprising that responses in adenine nucleotide pools correlate with tissue concentrations of BRH contaminants in exposed organisms. Measurement of the adenine nucleotide concentrations may help to characterize the energy costs incurred by organisms under stressful conditions.

PREFACE

This report describes work performed by the US Environmental Protection Agency (USEPA), Environmental Research Laboratory, Narragansett, R. I. (ERLN), as part of the Interagency Field Verification of Testing and Predictive Methodologies for Dredged Material Disposal Alternatives Program (Field Verification Program (FVP)). The FVP was sponsored by the Office, Chief of Engineers (OCE), US Army, and was assigned to the US Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss. The objective of this interagency program was to field verify existing predictive techniques for evaluating the environmental consequences of dredged material disposal under aquatic, intertidal, and upland conditions. The aquatic portion of the FVP was conducted by ERLN, with the wetland and upland option conducted by WES.

The principal investigators for this aquatic study and authors of this report were Drs. Gerald E. Zaroogian, ERLN; D. Michael Johns, Tetra Tech; Peter F. Rogerson and Gerald Hoffman, ERLN; and Mr. William G. Nelson, Science Applications International Corporation (SAIC). Laboratory-cultured algae were provided by Mr. Greg Tracey, SAIC. Technical support for the adenylate energy charge measurements was provided by Ms. Mary Johnson, ERLN. Diving support for the field portion of the study was provided by Messrs. Bruce Reynolds and Norman Rubinstein, ERLN, and Greg Tracey, SAIC.

Analytical chemistry support was provided by Mr. Richard Lapan, Mr. Curtis Norwood, and Mr. Frank Osterman, ERLN; Mr. Richard McKinney, Mr. Warren Boothman, Ms. Adria Elskus, Ms. Eileen McFadden, Mr. Lawrence LeBlanc, Mr. Robert Bowen, and Ms. Sharon Pavignano, SAIC; and Ms. Kathleen Schweitzer, University of Rhode Island.

Mses. Joan E. Seites, Barbara S. Gardiner, and Colette J. Brown, Computer Science Corporation (CSC), provided word processing support in the preparation of this report. Predictive models for field exposures were supplied by Drs. John F. Paul, ERLN, and Wayne R. Munns, SAIC. In addition, assistance in statistical analysis was provided by Dr. James Heltshe and Mr. Jeffery Rosen, CSC. Critical reviews of this report were completed by Drs. Eugene Jackim, John H. Gentile, and Gerald G. Pesch, ERLN. Technical reviews were provided by WES personnel.

The USEPA Technical Director for the FVP was Dr. Gentile; the Technical Coordinators were Dr. Pesch and Mr. Walter Galloway. The OCE Technical

Monitors were Drs. John Hall, Robert J. Pierce, and William L. Klesch.

The study was conducted under the direct WES management of Drs. Thomas M. Dillon and Richard K. Peddicord and under the general management of Dr. C. Richard Lee, Chief, Contaminant Mobility and Criteria Group; Mr. Donald L. Robey, Chief, Ecosystem Research and Simulation Division; and Dr. John Harrison, Chief, Environmental Laboratory. The FVP Coordinator was Mr. Robert L. Lazor, and the Environmental Effects of Dredging Program (EEDP) Managers were Mr. Charles C. Calhoun, Jr., and Dr. Robert M. Engler. Dr. Thomas D. Wright was the WES Technical Coordinator for the FVP reports. This report was edited by Ms. Jamie W. Leach of the WES Information Technology Laboratory.

COL Dwayne G. Lee, CE, was Commander and Director of WES. Dr. Robert W. Whalin was Technical Director.

This report should be cited as follows:

Zaroogian, G. E., Rogerson, P. F., Hoffman, G., Johnson, M., Johns, D. M., and Nelson, W. G. 1988. "A Field and Laboratory Study Using Adenylate Energy Charge as an Indicator of Stress in *Mytilus edulis* and *Nephtys incisa* Treated with Dredged Material," Technical Report D-88-4, prepared by the US Environmental Protection Agency, Narragansett, R. I., for the US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

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A FIELD AND LABORATORY STUDY USING ADENYLATE ENERGY CHARGE AS AN INDICATOR OF STRESS IN *MYTILUS EDULIS* AND *NEPHTYS INCISA* TREATED WITH DREDGED MATERIAL

PART I: INTRODUCTION

Background

1. The Marine Protection, Research, and Sanctuaries Act (Public Law 92-532) was passed by Congress in 1972. This law states that it is the policy of the United States to regulate disposal of all types of materials into ocean waters and to prevent or strictly limit disposal of any material that would adversely affect human health, welfare, the marine environment, or ecological systems. The implementation of this law, through the issuance of permits as defined in the final regulations and criteria, is shared jointly by the US Environmental Protection Agency (USEPA) and the US Army Corps of Engineers (CE).

2. In 1977, the CE and the USEPA prepared technical guidance for the implementation of the final ocean dumping regulations in the form of a manual entitled "Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters" (USEPA/CE 1977). This manual specified which test procedures were to be followed in collecting information to be used in making a disposal decision. Among the procedures were those for: (a) chemically characterizing the proposed dredged material; (b) determining the acute toxicity of liquid, suspended particulate, and solid phases; (c) estimating the potential contaminant bioaccumulation; and (d) describing the initial mixing during disposal. These methods have been used for determining the suitability of dredged material for open-water disposal. The procedures in this manual represented the technical state of the art at that time and were never intended to be inflexible methodologies. The recommended test methods were chosen to provide technical information consistent with the criteria specified in the regulations. However, use of the manual in the permit process has identified conceptual and technical limitations with the recommended test methods (Gentile and Scott 1986).

3. To meet this critical need, the Interagency Field Verification of

Testing and Predictive Methodologies for Dredged Material Disposal Alternatives Program, or the Field Verification Program (FVP), was authorized in 1982. This 6-year program was sponsored by the Office, Chief of Engineers, US Army, and was assigned to the US Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss. The objective of this interagency program was to field verify existing test methodologies for predicting the environmental consequences of dredged material disposal under aquatic, intertidal, and upland conditions. The aquatic portion of the FVP was conducted by the USEPA Environmental Research Laboratory, Narragansett, R. I. (ERLN). The intertidal and upland portions, conducted by WES, are reported in separate documentation.

4. The ERLN was responsible for conducting research on the aquatic option for disposal of dredged material. There were three research objectives for this portion of the program. The first was to demonstrate the applicability of existing test methods for detecting and measuring the effects of dredged material and to determine the degree of variability and reproducibility inherent in the testing procedure. This phase of the program (Laboratory Documentation) is complete, and the results have been published in a series of technical reports. This information provides insight into how the various methods function, their sources of variability, their respective and relative sensitivities to the specific dredged material being tested, and the degree of confidence that can be placed on the data derived from the application of the methods.

5. The second objective was to field verify the laboratory responses by measuring the same responses under both laboratory and field exposures. A basic and often implicit assumption is that results derived from laboratory test methods are directly applicable in the field. While this assumption is intuitive, there are no supporting data from studies on complex wastes in the marine environment. The study reported herein offers a unique opportunity to test this basic assumption.

6. The third objective was to determine the degree of correlation of tissue residues resulting from bioaccumulation of dredged material contaminants with biological responses from laboratory and field exposure to dredged material. However, this study was not designed to address cause-effect relationships, and the multicontaminant nature of the dredged material precludes any such assumptions.

7. The aquatic disposal portion of the FVP was a site- and waste-specific case study that applied the concepts and principles of risk assessment. The disposal site for the FVP was a historical site known as the Central Long Island Sound (CLIS) disposal site (1.8 by 3.7 km) located approximately 15 km southeast of New Haven, Conn. (Figure 1). The sedimentology at

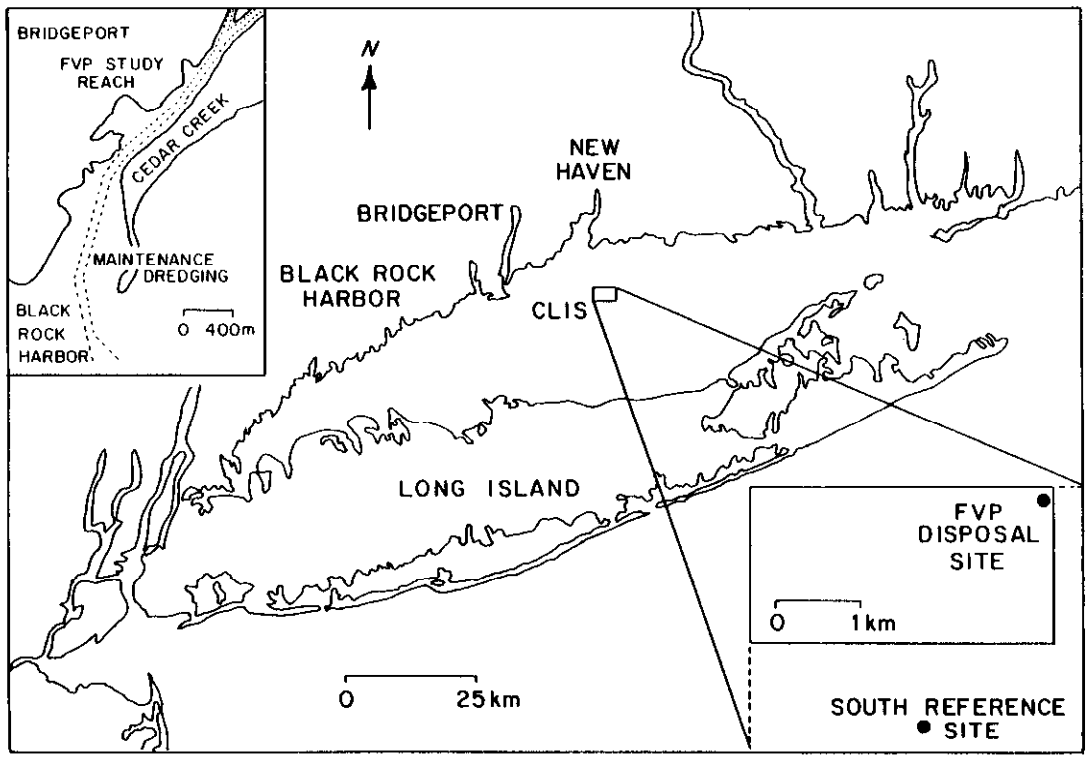

Figure 1. Central Long Island Sound disposal site and Black Rock Harbor dredge site

the disposal and reference sites is primarily silt-clay, with a mean grain size of 0.013 mm. Thermal stratification occurs from April to September, and during this period bottom salinity is slightly higher than that of the surface. Tidal currents typically dominate the near-bottom water in an east-west direction. Suspended sediment concentrations average 10 mg/ℓ, with storm-induced values to 30 mg/ℓ. The baseline community data revealed a homogeneous, mature infaunal community dominated by the polychaete *Nephtys incisa* and the bivalve molluscs *Nucula proxima* and *Yoldia limatula*.

8. The FVP disposal site was selected within the CLIS so as to minimize contamination from other sources, including relic disposal operations or

ongoing disposal activities occurring during the study period. This was necessary to ensure a point source of contamination. The uniformity of physical, chemical, and biological properties of the disposal site prior to disposal allowed detection of changes in these properties due to the disposal of the dredged material. Finally, the stations used to study the biological effects in this study were selected along the primary axis of current flow to represent a gradient of potential exposure for the biota (Figure 2).

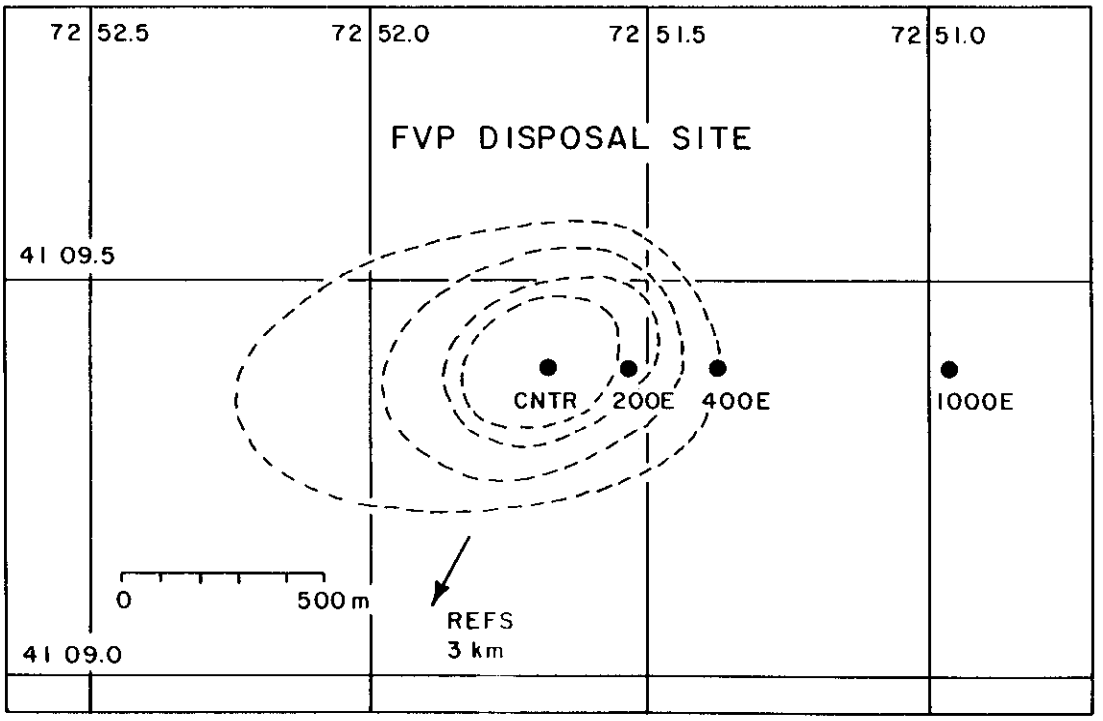

Figure 2. FVP sampling stations

9. The spatial scale of this study was near-field and limited to the immediate vicinity of the disposal site. A primary assumption was that the mound of dredged material constituted a point source of contamination. The temporal scale for the study was 4 years, which included a year of predisposal data collection to define seasonal patterns in the physical, chemical, and biological variables and 3 years of postdisposal data collection to address the objectives of the program and to evaluate the long-term impacts of the disposal operation on the surrounding benthic communities.

10. The dredging site was Black Rock Harbor (BRH), located in Bridgeport, Conn., where maintenance dredging provided a channel 46 m wide and 5.2 m deep at mean low water (Figure 1). Approximately 55,000 m^3 of material

was dredged during April and May 1983 and disposed in 20 m of water in the northeastern corner of the CLIS disposal site.

11. The dredged material from BRH contained substantial concentrations of both organic and inorganic contaminants (Rogerson, Schimmel, and Hoffman 1985). Polychlorinated biphenyls (PCBs) were present in the dredged material at a concentration of 6,400 ng/g, and polynuclear aromatic hydrocarbons (PAHs) with molecular weights between 166 and 302 were present at concentrations ranging from 1,000 to 12,000 ng/g, respectively. Alkyl homologs of the PAHs were also present in the dredged material at concentrations between 1,000 and 13,000 ng/g. Inorganic contaminants of toxicological importance present in the dredged material included copper (2,900 µg/g), chromium (1,480 µg/g), zinc (1,200 µg/g), lead (380 µg/g), nickel (140 µg/g), cadmium (24 µg/g), and mercury (1.7 µg/g).

Project Scope

12. The FVP was unique among marine research studies for several reasons. The program objectives were directly focused on addressing specific limitations in the methodologies and interpretive framework of the current regulatory process. Among the program strengths were: (a) a suite of biological endpoints using the same material was developed and evaluated; (b) the biological tests represented different levels of biological organization; (c) the tests were conducted under both laboratory and field exposure conditions; (d) the tissue residues were examined concurrently with measurements of biological effects; (e) the duration of the study was adequate to evaluate the use of community responses as a benchmark against which other biological responses could be compared; and (f) the project was a site- and waste-specific case study for the application and evaluation of the components of a risk assessment, including the development of methodologies for predicting and measuring field exposures in the water column and benthic compartments. Limitations of this study were: (a) only one dredged material was evaluated, which constrained certain types of comparisons; (b) the size of the study put limits on the extent to which any given objective could be examined; and (c) the resources allocated to determine field exposures were limited. The latter constraint was particularly important because the laboratory-field comparisons and the risk assessment process both required accurate predictions of environmental exposures.

Laboratory-to-Field Comparisons

13. The field verification of laboratory test methods was designed to compare the exposure-response relationships measured in both the laboratory and the field. Exposure for the purposes of this discussion includes the total dredged material with all of its contaminants. Specific contaminants are used as "tracers" to verify the exposure environment, which is described in terms of BRH dredged material, and to illustrate exposure-response relationships between the laboratory and the field. The specific contaminants are a subset of a comprehensive suite of chemicals analyzed in this study and were selected based upon their environmental chemistry and statistical representativeness. The use of specific contaminants in no way implies a cause-and-effect relationship between contaminant and response.

14. Exposure in open marine systems is characterized by highly dynamic temporal and spatial conditions and cannot be completely replicated in laboratory systems. Consequently, the approach chosen for this program was to develop laboratory exposure-response data using only general field exposure information.

Residue-Effects Relationships

15. Determining the relationship between contaminant tissue residues resulting from bioaccumulation and the biological responses measured is a principal objective of this program. Such relationships do not in any way imply cause and effect, but rather seek to determine the statistical relationship between an effect and any associated residues. The approach used is to determine specific contaminant residues in the tissues of the organisms as the result of exposure to the whole dredged material in both the laboratory and the field. These residues are determined at the same time that biological responses are being measured. Residue-effect relationships will be described and interpreted for both laboratory and field exposures.

Adenylate Energy Charge

16. Atkinson and Walton (1967) proposed adenylate energy charge (AEC) as a measure of energy potentially available from the adenylate system for

cell metabolism. The AEC defined as (ATP + 1/2 ADP)/(ATP + ADP + AMP),* has a maximum value of 1.0 when all adenylate is in the form of ATP, and a minimum value of 0 when all adenylate is in the form of AMP (Atkinson and Walton 1967). The energy charge is considered important in the control of key catabolic and anabolic pathways (Atkinson 1971). Values of energy charge correlate with physiological condition: energy charges between 0.8 and 0.9 are typical of organisms that are actively growing and reproducing, usually under optimal environmental conditions (Atkinson 1971; Chapman, Fall, and Atkinson 1971; Rainer, Ivanovici, and Wadley 1979; Ivanovici 1980b). Values in the range of 0.5 to 0.7 have been observed in organisms that are stressed (Ball and Atkinson 1975; Behm and Bryant 1975; Wijsman 1976; Rainer, Ivanovici, and Wadley 1979; Ivanovici 1980b) and whose growth and reproductive rates are reduced (Chapman, Fall, and Atkinson 1971). Values below 0.5 have been associated with irreversible loss of viability under detrimental conditions (Ridge 1972; Montague and Dawes 1974; Skjoldal and Bakke 1978).

17. In this report the responses of adenine nucleotides and AEC to stress are considered. The central role of adenine nucleotides in energy transformation and in metabolic regulation suggests their potential usefulness as indicators of sublethal stress. These responses were measured in the mussel *Mytilus edulis* and the polychaete *Nephtys incisa* exposed to a contaminated dredged material, BRH sediments, both in the laboratory and in the field.

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* ATP = adenosine triphosphate, ADP = adenosine diphosphate, and AMP = adenosine monophosphate.

PART II: MATERIALS AND METHODS

Laboratory Methods

Sediment collection

18. Two sediment types were used to conduct laboratory tests for the field verification studies. The reference (REF) sediment was collected from the South Reference site in Long Island Sound (40°7.95' N and 72°52.7' W) by Smith-MacIntyre grab (0.1 m^2), press sieved through a 2-mm sieve, and stored at 4° C until used. Prior to dredging, contaminated sediment was collected from BRH (41°9' N and 73°13' W) with a gravity box corer (0.1 m^2) to a depth of 1.21 m, thoroughly mixed, press sieved through a 2-mm sieve, and refrigerated (4° C) in barrels until used. Details of sediment collection and storage procedures may be found in Rogerson, Schimmel, and Hoffman (1985).

Organism collection and holding

19. *Mytilus edulis.* Two separate experiments were completed using oxidized REF and BRH sediments. Mussels were collected from the Narragansett Bay reference population (71°24.0' W by 41°29.4' N) with a scallop dredge from a depth of 10 m. Collection information for each experiment is listed in Table 1. The animals were sorted to obtain a size range of 50- to 55-mm shell length and acclimated in flowing unfiltered Narragansett Bay seawater at a rate of 1° C per day to 15° C.

Table 1

Collection Information for the *M. edulis* Used in the Laboratory Experiments

Experiment	Collection Date	Experiment Begun	Temperature °C	Salinity g/kg
1	17 Jan 85	05 Feb 85	2.0	30.0
2	22 Feb 85	12 Mar 85	5.0	30.0

20. *Nephtys incisa.* *Nephtys incisa* for laboratory studies were collected with a Smith-MacIntyre grab sampler (0.1 m^2) at the South Reference site (Figure 1). Collection information for each experiment is listed in Table 2. The sediment containing the *N. incisa* was brought to the laboratory where it was sieved and the *N. incisa* were picked and sorted by size. Tests

Table 2

Collection Information for the *N. incisa* Used in the Laboratory Experiments

Experiment	Duration days	Collection Date	Experiment Start Date	Temperature °C	Salinity g/kg
1	10	30 Oct 84	10 Dec 84	15	28.0
2	28	27 Feb 85	12 Mar 85	1	28.5
3	42	23 Apr 85	3 May 85	10	29.3

were conducted with adult specimens. These individuals were placed in REF sediment, in flowing seawater, and were acclimated at a rate of 1° C per day to 20° C. They were fed powdered prawn flakes, ad libitum, during this period.

Suspended sediment dosing system

21. Laboratory studies required the construction of two identical sediment dosing systems to provide simultaneously either BRH or REF material as suspended sediment. Each dosing system (Figure 3) consisted of a conical-shaped slurry reservoir placed in a chilled fiberglass chamber, a diaphragm pump, a 4-ℓ separatory funnel, and several return loops that directed the

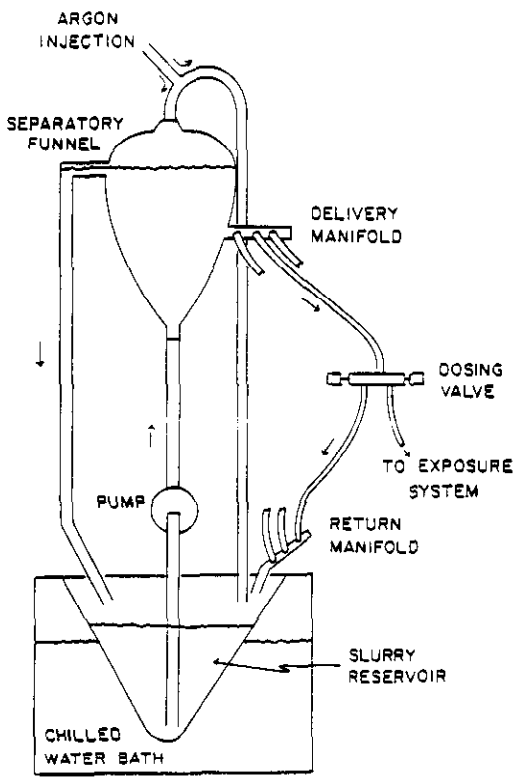


Figure 3. Suspended sediment dosing system

particulate slurry through dosing valves. The slurry reservoirs (40 cm in diameter by 55 cm high) contained 38 ℓ of slurry composed of 36 ℓ of filtered seawater and 2 ℓ of either BRH or REF sediment. The fiberglass chamber (94 cm by 61 cm by 79 cm high) was maintained between 4° and 10° C using an externally chilled water source to minimize microbial degradation during the test. Polypropylene pipes (3.8 cm diam) extended to the bottom of the reservoir cones and were connected to pumps (16- to 40-ℓ/min capacity) fitted with Teflon diaphragms. These pumps were used to circulate the slurry while minimizing abrasion that might produce changes in the physical properties (e.g., particle size) of the material.

22. The slurry was pumped up to separatory funnels and returned via an overflow to the reservoir through polypropylene pipes. The separatory funnel provided the constant head pressure needed to circulate the slurry through Teflon tubing to the dosing valves where the slurry was mixed with seawater to provide the desired concentrations for the toxicity tests. Narragansett Bay seawater filtered (to 15 μ) through sand filters was used.

Suspended sediment oxidation system

23. The REF and BRH sediments used in these experiments were oxidized prior to introduction into the dosing system. The objective of this portion of the FVP was to evaluate the relationship between biological endpoints measured in the laboratory and the field. The field collections of sediment indicated rapid oxidation of the surficial BRH sediments on the disposal mound. Because the most likely source of particulate contaminants in the water column was the oxidized surficial sediment, it was decided that laboratory exposures would be conducted with BRH sediment that had been oxidized in a consistent manner.

24. In order to obtain consistent states of oxidation for both REF and BRH sediments, 2 ℓ of sediment was transferred to an inverted polycarbonate carboy and diluted to 19 ℓ with filtered natural seawater at room temperature and aerated for 3 to 4 days (Figure 4). The contents were transferred to the composite dosing system reservoir and diluted to 38 ℓ with natural seawater. Chemical oxygen demand measurements indicated that this time period was sufficient to satisfy the immediate oxygen demand of the sediments.

Exposure system

25. *Mytilus edulis.* An exposure system was constructed to provide a constant concentration of suspended sediment to mussels in the laboratory.

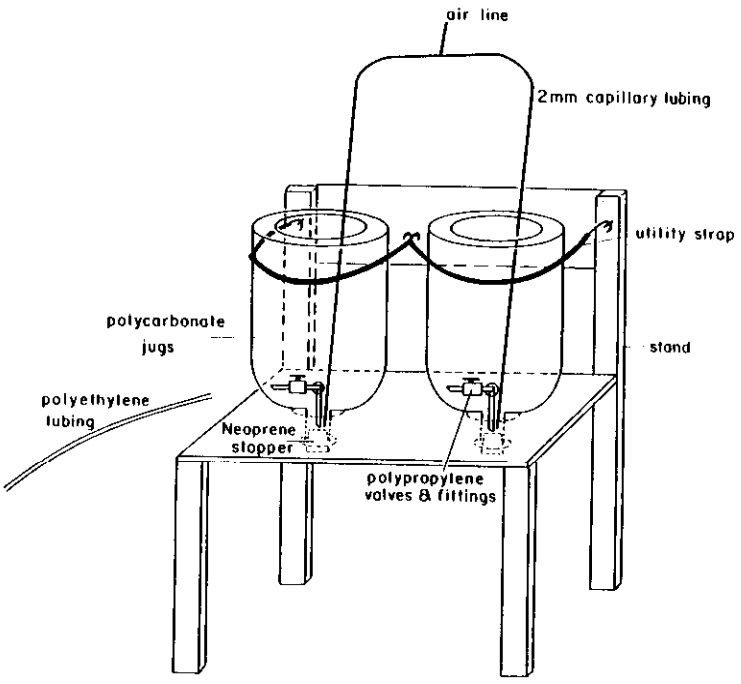


Figure 4. Suspended sediment oxidation system

This system consisted of recirculating loops from the suspended sediment dosing system connected to a dosing valve at each exposure chamber. The concentration of total suspended particulates was maintained at approximately 12 mg/ml in both the REF and BRH loops. The exposure system was capable of delivering either REF or BRH sediment directly into each mussel exposure chamber via a dosing valve. The combined use of a REF and a BRH dosing valve at an exposure chamber allowed delivery of a mixture of the two sediments. The percent concentrations of BRH and REF sediment varied between treatments; however, a total suspended sediment concentration of approximately 10 mg/ℓ (dry weight) was maintained in all five laboratory exposure treatments. This concentration was chosen because it approximated the background field suspended sediment concentration present at the CLIS disposal site.

26. Each mussel exposure chamber was equipped with a transmissometer, an instrument capable of measuring light attenuation due to suspended sediment in the chamber (Figure 5). The dosing valves for each treatment were controlled by a transmissometer-microprocessor feedback loop (Sinnett and Davis 1983). The transmissometer in each chamber was calibrated by regressing suspended sediment concentrations, measured by filtration onto glass fiber filters, with the transmissometer units displayed on a microprocessor. A transmissometer value was calculated that corresponded with the desired suspended

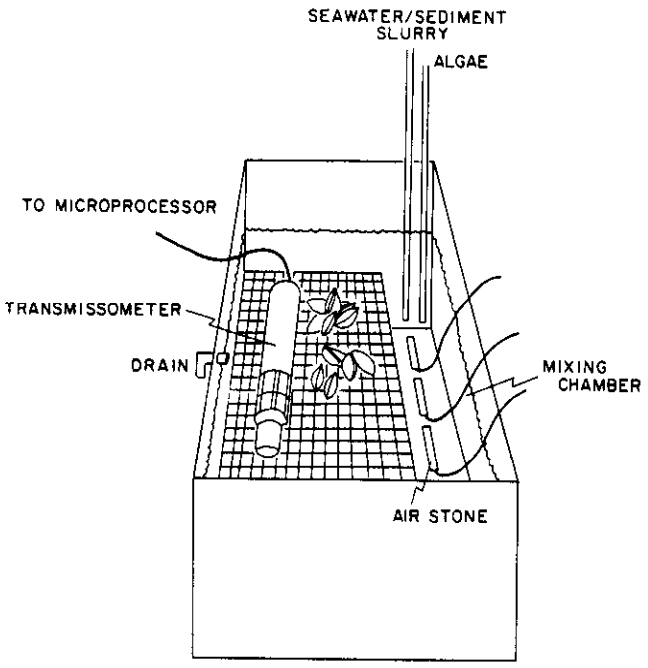


Figure 5. Laboratory exposure system for *M. edulis*

sediment concentration of 10 mg/ℓ for each chamber. As the mussels removed suspended sediments, the microprocessor opened dosing valves to deliver additional suspended sediment at 2-min intervals. In this manner, suspended sediment concentrations were maintained at the desired values (±10 percent). The transmissometer circuit was also connected to a strip chart recorder, which allowed the operation of the system to be monitored continuously. Each chamber was aerated with three 25- by 2.5-cm air stones to provide sufficient oxygen and to ensure even distribution of suspended particulates (Figure 5).

27. In addition to the suspended sediment, food in the form of a unicellular alga, *Isochrysis galbana*, was supplied to each exposure chamber. Periodic measurements were made of mussel clearance rates in each chamber to determine the volume of algae required to maintain an algal concentration of 0.5 mg/ℓ. This concentration constituted an adequate maintenance ration for the mussels. Algae were added at 5-min intervals by means of a peristaltic pump. All experiments were conducted at 15° C with filtered seawater that flowed through each experimental chamber at a rate of 0.4 ℓ/min. Each chamber was cleaned every other day.

28. The purpose of the laboratory experiments was to expose *M. edulis* to a range of BRH concentrations that may have been present in CLIS and to assess the biological effect on these organisms. *Mytilus edulis* were exposed for approximately 1-month periods at the CLIS disposal site; therefore,

exposures of similar duration, 28 days, were used for the laboratory exposures.

29. At the start of both experiments, 150 mussels were placed into each chamber. *Mytilus edulis* were sampled at time zero for determination of initial tissue residue concentrations and for adenine nucleotide measurements.

30. Experiment 1 consisted of three exposure treatments: 100-, 50-, and 0-percent BRH suspended sediment. *Mytilus edulis* were removed from each treatment on day 14 for chemical and biological analysis. Experiment 1 was terminated at day 14 because adverse biological effects (e.g., reduced filtration rate) were observed in both treatments containing BRH sediment.

31. Experiment 2 was conducted with lower concentrations of BRH suspended sediment. Exposure treatments of suspended sediment in Experiment 2 were 30-, 10-, and 0-percent BRH. Fifteen organisms were removed on days 7, 14, 21, and 28 for tissue residue analysis. Whole water chemistry samples were taken within 1 day of organism sampling. Dissolved and particulate water samples were taken for chemical analysis within 24 hr of days 0, 14, and 28. Mussels were sampled on days 14 and 28 for biological analysis. In addition, a water sample was taken on day 29 to evaluate the performance of the system without any mussels in the exposure chamber.

32. The operation of the system (dosing valves, flow rates, etc.) was monitored daily. Experiments using the 100- and 0-percent BRH treatment required only one dosing valve each, while the 50-percent BRH treatment required a REF and BRH valve that delivered equal amounts of suspended material. A strip chart record for each treatment indicated that the dosing valves were operating properly. The 10- and 30-percent BRH treatments also required two dosing valves per treatment; however, the REF and BRH dosing valves delivered different amounts of suspended material. This was accomplished by adjusting the delivery volume of each valve. The mixture of BRH and REF material was checked daily and adjusted if necessary.

33. Nephtys incisa. In the laboratory tests with *N. incisa*, the dosing system was set to maintain nominal concentrations of 200 mg/ℓ (dry weight) of suspended sediments with seawater flow rates producing five volume replacements per exposure chamber per day. These flow rates meet the minimum recommended by the American Society for Testing and Materials (1980) and were intended to maximize residence time of the suspended sediments in the exposure chambers.

34. A suspended sediment proportional diluter (Figure 6) was used to mix the small quantities of concentrated sediment slurries (10 to 20 g/ℓ from the sediment dosing system with filtered seawater to produce dilute sediment suspensions in the milligrams-per-litre range. It then combined slurries of different types (e.g., REF and BRH sediment suspensions) proportionally to maintain the same concentration of suspended sediment with different ratios of the two sediments.

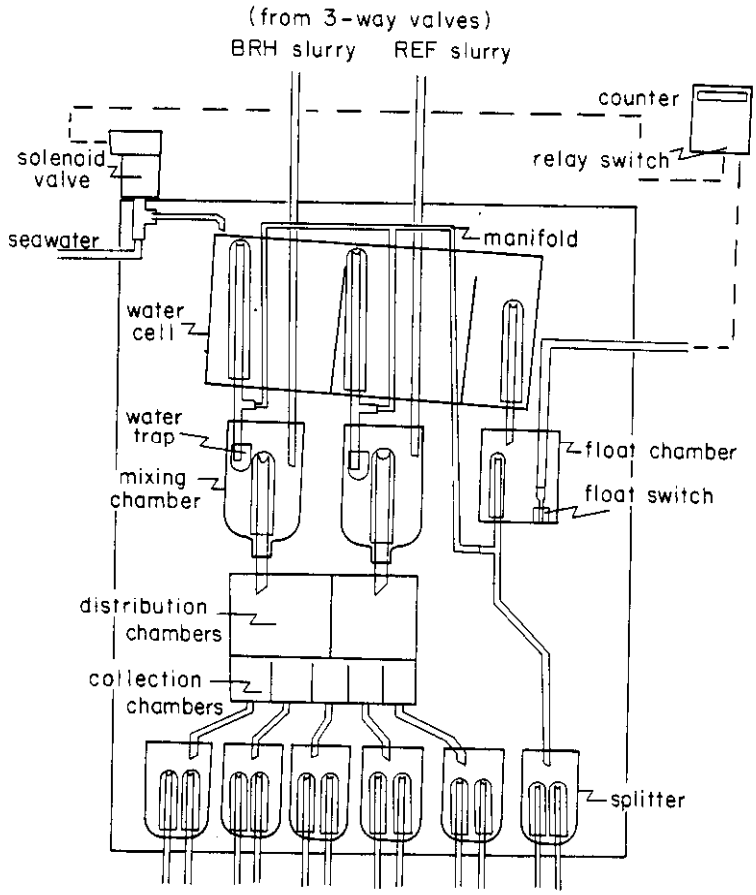


Figure 6. Proportional diluter used to deliver suspended sediment to the *N. incisa* exposure chambers

35. The exposure chamber for *N. incisa* is illustrated in Figure 7. Polycarbonate bottles (19 ℓ) used commercially for shipping spring water were cut off at the top. REF sediment (2 ℓ/chamber) was added to a depth of 4 cm, and Plexiglas strips were inserted into the sediment, dividing it into pie-shaped sections. This permitted subsampling without disturbing the entire chamber. Each chamber was filled with filtered seawater at 20° C. After the sediment in the chambers was permitted to settle and equilibrate for about

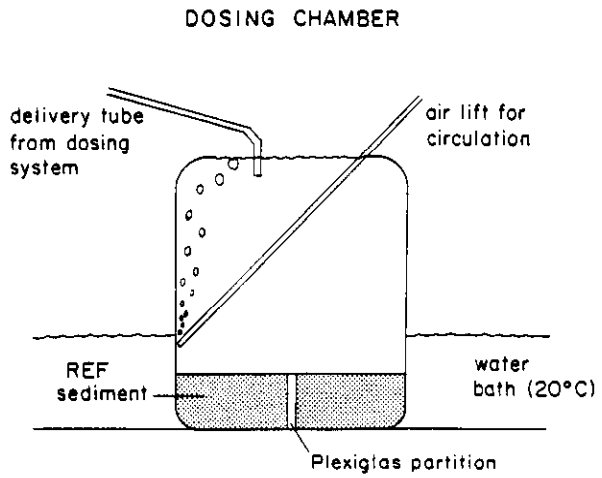


Figure 7. *Nephtys incisa* exposure chamber

4 hr, *N. incisa* were added, and an additional 2 hr was allowed for the worms to burrow into the sediment. The delivery tubes from the proportional diluter were then put in place, and a low pressure airlift was turned on to keep the dosed sediments in suspension. This system allowed very little sediment deposition during the course of experiments. Excess seawater was permitted to overflow the brim of each chamber. Earlier experiments indicated that once the worms burrowed into clean REF sediment, they would not attempt to escape. Therefore, the chamber design used here was considered acceptable. Two chambers were used for each of the three treatments for a total of six chambers per treatment. The two chambers did not represent replicates, but were used to accommodate enough worms for chemical and biological analysis in each experiment.

36. Three experiments were conducted during this phase of the FVP in which adenylate nucleotide measurements were made. These experiments lasted 10, 28, and 42 days, respectively, and had exposure conditions of 100-, 50-, and 0-percent BRH suspended sediment. The 42-day experiment provided time series sampling for the three exposure conditions. Worms were removed at time 0, day 28, and day 42. This experiment was supported with chemical analyses of the seawater and of the *N. incisa*. *Nephtys incisa* were collected on a sieve after removal of a pie-shaped aliquot of bedded sediment from each chamber. Clean REF sediment, without *N. incisa*, was returned to the vacated section to maintain the integrity of the exposure chamber.

37. Suspended sediment, temperature, and salinity were measured routinely during each experiment. Dissolved oxygen (DO) concentrations were not expected to be a problem because of the large volume of the chamber and the

use of an airlift. However, DO levels were determined once during each experiment and never differed significantly from expected saturation levels. The worms were fed 100 mg of powdered prawn flakes per chamber per day for the duration of each experiment.

Adenylate nucleotide extraction

38. *Mytilus edulis.* The adductor muscle was rapidly dissected out, blotted dry, placed on a labelled polyethylene strip (Gladwrap®), and freeze clamped with aluminum blocks cooled to -196° C with liquid nitrogen (Bergmeyer 1965; Ivanovici 1980a). The time between sampling and dissection never exceeded 10 min. Tissue samples were removed and freeze clamped in less than 30 sec, and the labelled samples were stored in liquid nitrogen until homogenization.

39. Adenine nucleotides were extracted from tissues with a method similar to that of Ivanovici (1980a) (Figure 8). The freeze-clamped tissue was quickly transferred from its wrapping to a tared stainless steel homogenizing tube previously cooled in liquid nitrogen and placed in a polyurethane insulator and weighed. Tissue samples (approximately 0.2 g) were ground to a fine powder at -196° C. Perchloric acid (PCA) (1 ml, 6 percent v/v) was added to the ground tissue, allowed to freeze, ground to a powder, and mixed with the tissue sample. This mixture was kept on ice and allowed to thaw, after which additional ice-cold PCA was added (the final ratio of tissue to PCA was 1:10, w/v) and then centrifuged at 5° C and 6,000 g's for 20 min after thorough mixing. The supernatant was decanted into a (polyethylene) centrifuge tube containing 5 µℓ of universal indicator and adjusted to pH 6.5 to 7.0 with solid K_2CO_3. These tubes were left on ice for approximately 15 min to allow CO_2 evolution and then centrifuged as above. The supernatant was decanted from the $KClO_4$ precipitate into clean (polyethylene) centrifuge tubes and assayed or stored at -20° C. Generally, 20 samples were prepared each day. Extraction efficiencies of the adenine nucleotides from adductor tissue muscle tissue of *M. edulis* by PCA were consistently greater than 92 ± 0.5 percent.

40. *Nephtys incisa.* Worms collected on a fine mesh sieve (0.9-mm mesh) were immediately anesthetized by immersion of sieve and worms into a 7-percent solution of $MgCl_2$ in seawater for 2-1/2 min (Dean and Mazurkiewicz 1975). The worms were washed by immersion of the sieve in clean seawater, and the worms were then removed from the sieve and placed into a Carolina dish (75 mm diam) containing approximately 50 ml clean seawater. One or two anesthetized worms

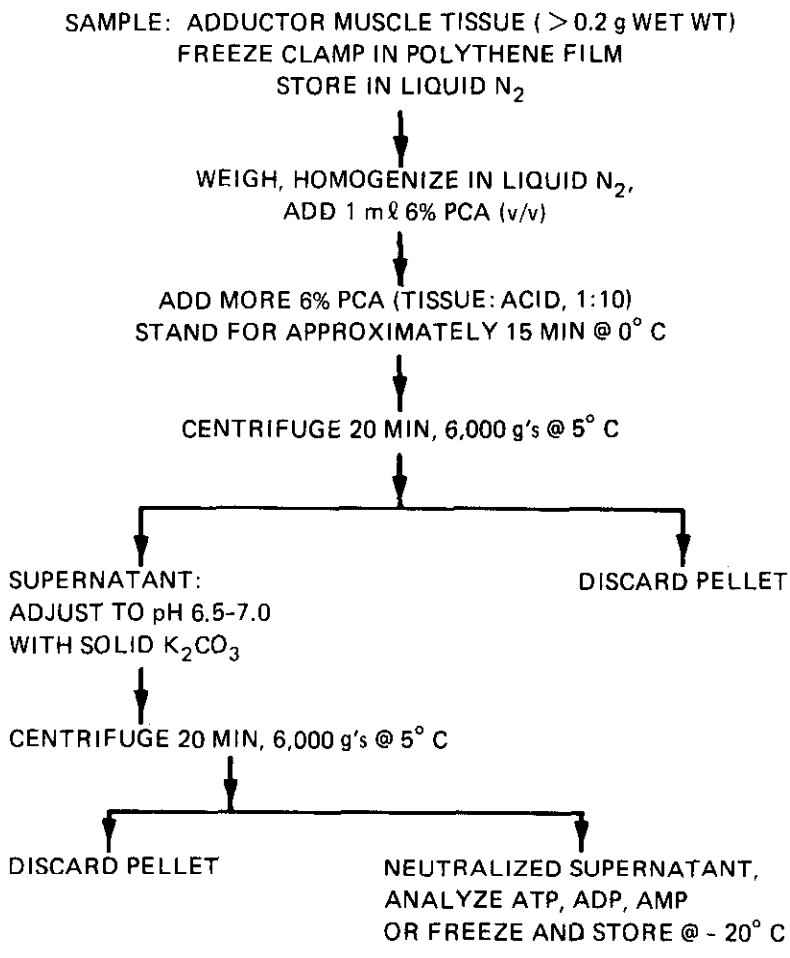


Figure 8. Summary of procedure for the extraction of adenine nucleotides from the adductor muscle of *M. edulis*

(>0.1-g wet weight) were placed on a millipore filter pad (25 mm, 1.2 μ), and as much seawater as possible was removed by vacuum. The anesthetized worms were gently removed from the filter pad onto a labelled polyethylene strip (Gladwrap®) and freeze clamped with aluminum blocks cooled by nitrogen to -196° C (Bergmeyer 1965; Ivanovici 1980a).

41. Adenine nucleotides were extracted from tissues with a method similar to that of Ivanovici (1980a) (Figure 9). The freeze-clamped tissue was quickly transferred from its wrapping to a tared stainless steel homogenizing tube previously cooled in liquid nitrogen and placed in a polyurethane insulator and weighed. Tissue samples (approximately 0.1 g) were ground to a fine powder at -196° C. One half the total volume required of PCA (6 percent v/v) containing 0.33 percent ethylenediamine tetra-acetic acid (EDTA) (w/v) was

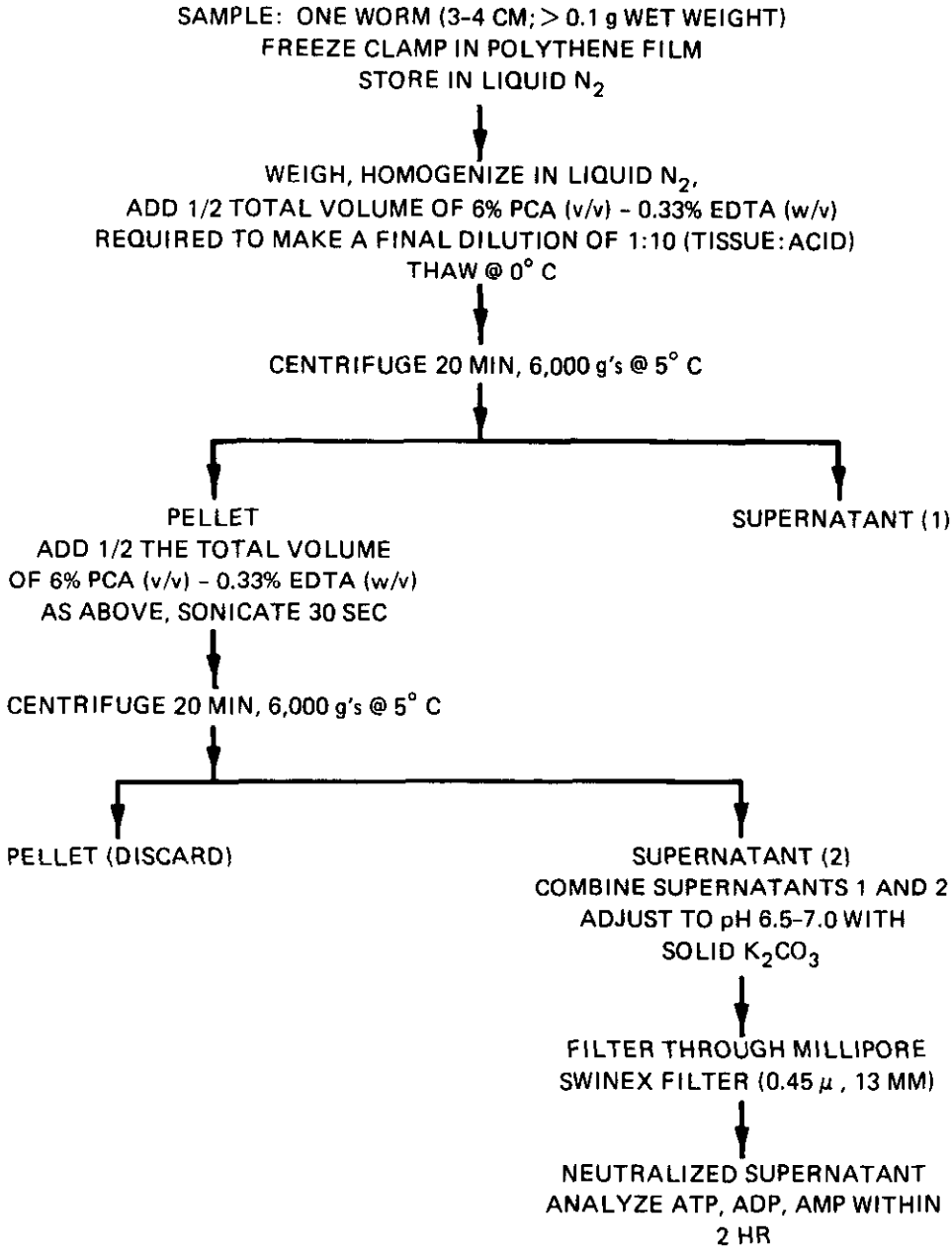


Figure 9. Summary of procedure for the extraction of adenine nucleotides from the tissue of *N. incisa*

added to the ground tissue, allowed to freeze, and ground to a powder with the tissue sample. This mixture was kept on ice and allowed to thaw. After thawing, the mixture was centrifuged at 5° C and 6,000 g's for 20 min after thorough mixing. The supernatant was decanted into a polyethylene centrifuge tube. Each pellet was extracted again with one half the total volume of PCA (6 percent v/v) - EDTA (0.33 percent w/v) required to make a final dilution of 1:10 (tissue:acid) and followed by sonication and centrifugation. The

supernatants were combined, and 5 µℓ of universal indicator was added and adjusted to pH 6.5 to 7.0 with solid K_2CO_3. These tubes were left on ice for approximately 15 min to allow CO_2 evolution and then centrifuged as above. The supernatant was decanted from the $KClO_4$ precipitate into clean polyethylene centrifuge tubes and assayed or stored at -20° C. Generally, 20 samples were prepared each day. Recovery efficiency of the extraction was determined by spiking tissue samples with ATP, ADP, and AMP, and recovery was calculated by the following equation:

$$\% \text{ Recovery} = \frac{(\text{Sample} + \text{Standard}) - \text{Sample}}{\text{Standard}} \times 100 \qquad (1)$$

where

Sample + Standard = concentration of adenylates in sample spiked with adenylates

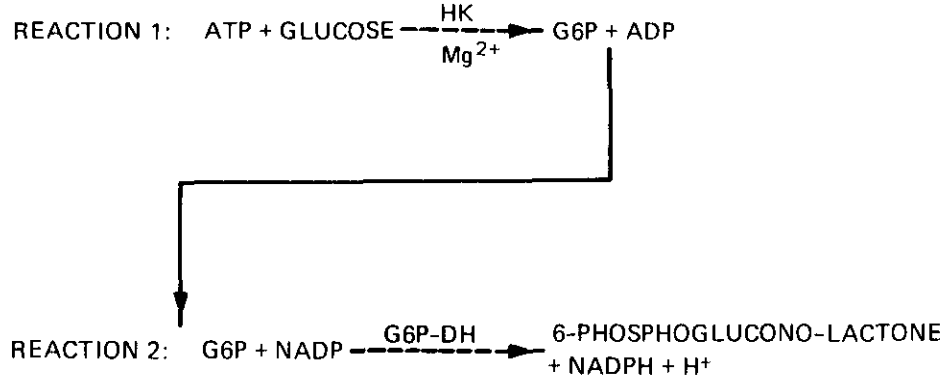
Sample = concentration of adenylates in samples

Standard = concentration of adenylate standard

Adenylate assay

42. *Mytilus edulis.* The concentrations of ATP, ADP, and AMP were determined spectrophotometrically (340 nm) with hexokinase (Lamprecht and Trautschold 1974), pyruvate kinase, and myokinase (Adam 1963), respectively (Figure 10). All enzymes, chemicals, and reagents (analytical grade) were obtained from Boehringer Mannheim, Indianapolis, Ind.

43. The principle of the ATP assay is as follows: glucose is phosphorylated by ATP to glucose-6-phosphate (G6P) with hexokinase (HK) (reaction 1). Glucose-6-phosphate then reacts with nicotinamide-adenine dinucleotide phosphate (NADP) to form 6-phosphoglucono-lactone and reduced nicotinamide-adenine dinucleotide phosphate (NADPH). This reaction is catalyzed by glucose-6-phosphate dehydrogenase (G6P-DH) (reaction 2).



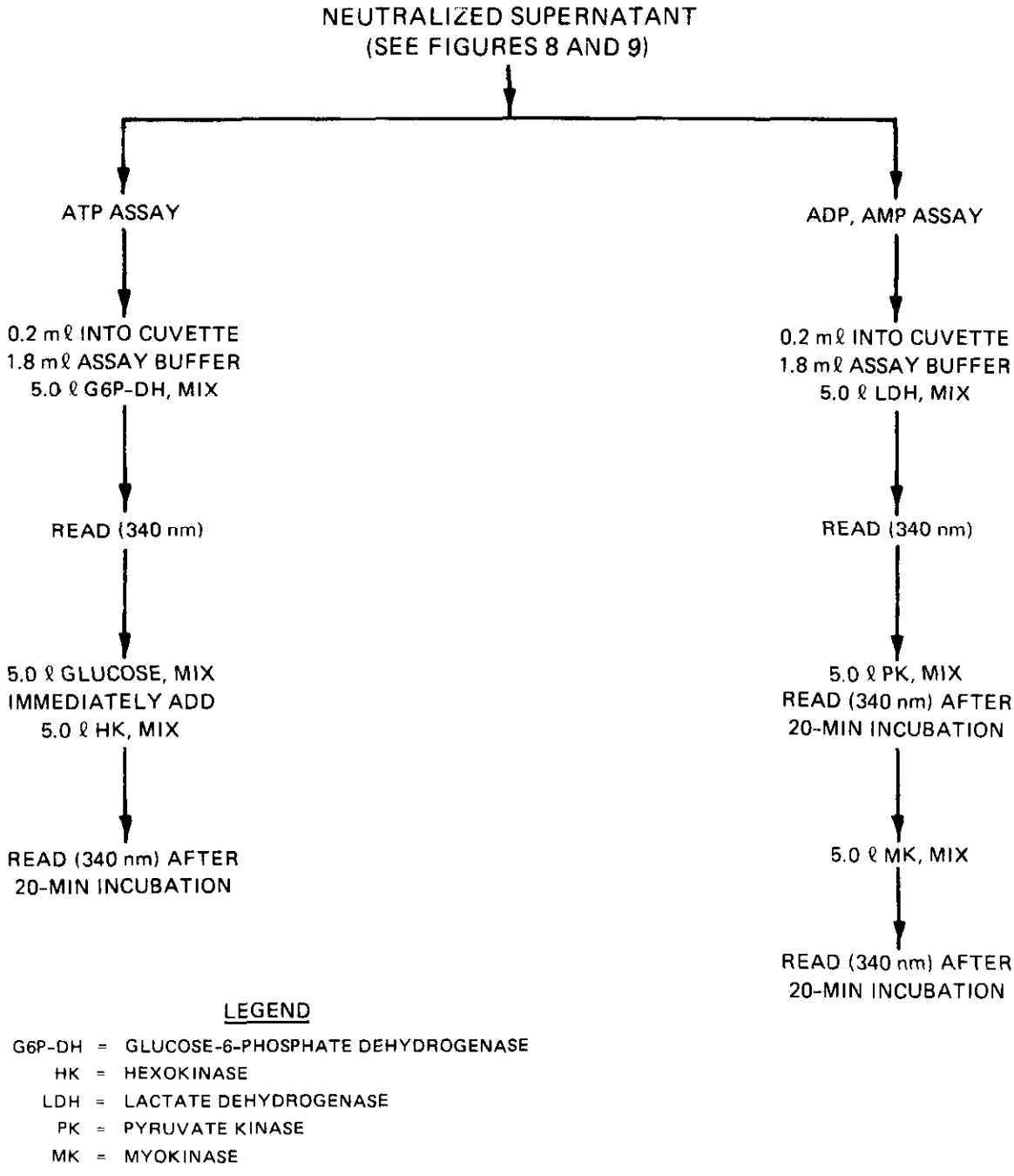
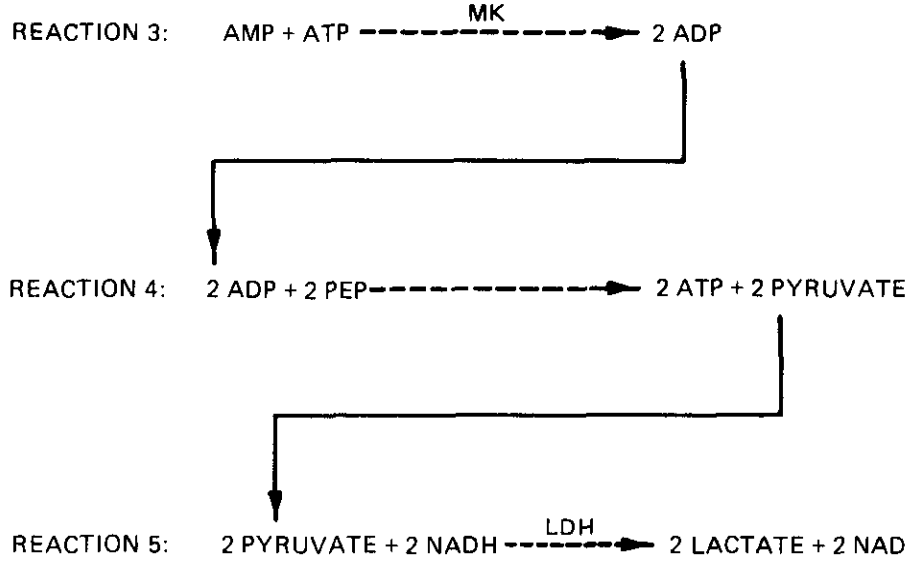


Figure 10. Summary of procedures for analysis of ATP, ADP, and AMP in adductor muscle tissue of *M. edulis* and whole *N. incisa*

Thus for every micromole of ATP, 1 µmol of NADPH is formed and causes an increase in absorbancy at 340 nm.

44. The principle of the ADP and AMP assays is as follows: pyruvate kinase (PK) catalyzes the phosphorylation of 1 µmol of ADP by phosphoenolpyruvate (PEP) to form 1 µmol of ATP and pyruvate (reaction 4). Pyruvate in turn

is converted to lactate by lactate dehydrogenase (LDH). Thus, 1 µmol of ADP results in the formation of 1 µmol of nicotinamide-adenine dinucleotide (NAD) (reaction 5). The decrease in absorbancy at 340 nm caused by the formation of NAD from NADH is, therefore, proportional to the amount of ADP present in the sample. After this absorbance change has been measured in a sample, myokinase (MK) is added. This enzyme catalyzes the formation of 2 µmol of ADP from 1 µmol each of AMP and ATP (reaction 3). In turn, 2 µmol of NAD are formed (reactions 4 and 5).



45. To determine if any inhibitory effects of neutralized tissue extracts on the nucleotide assay system occurred, known amounts of ATP, ADP, and AMP were added to neutralized extracts as internal standards and assayed to check for inhibitory or enhancement effects by the extract. The following equations were used to calculate correction factors (Cf):

$$X\% = \frac{(\text{Sample} + \text{Internal Standard}) - \text{Sample}}{\text{Internal Standard}} \qquad (2)$$

$$Cf_{\text{ATP, ADP, or AMP}} = \frac{100\%}{100\% + X\%} \qquad (3)$$

46. A Cf was not required for ATP since extracts of *M. edulis* adductor muscle had a negligible effect on absorbance. However, these same extracts increased absorbance, which caused high readings for ADP (112 percent) and AMP

(111 percent). Thus, a CF was required for ADP (0.89) and AMP (0.90) to calculate accurately their concentration.

47. *Nephtys incisa.* Within 2 hr after the final centrifugation, *N. incisa* tissue extracts were assayed for adenylate using a technique identical to that for *M. edulis* (Figure 10).

Field Methods

Organism collection and holding

48. *Mytilus edulis.* All mussels used in the field studies for the FVP were collected by scallop dredge from Narragansett Bay. In general, *M. edulis* were collected 1 to 2 days prior to field deployment to Long Island Sound. They were returned to the laboratory where 100, 5- to 7-cm organisms were sorted and placed into each polyethylene basket. All baskets were placed in holding tanks of flowing unfiltered seawater until deployed in the field.

49. *Nephtys incisa.* *Nephtys incisa* for field studies were collected at stations REFS, 1000E, 400E, 200E, and CNTR. Station locations were marked with buoys for the duration of this project. While the boat was anchored, a Smith-MacIntyre grab sampler (0.1 m^2) was used to collect bottom sediments. These sediments were wet sieved on deck (nested sieves of 2- and 0.5-mm mesh size), and organisms were collected. On each sampling date, *N. incisa* were collected for biological measurements. Specimens for adenylate measurements were prepared immediately on the boat.

Exposure

50. *Mytilus edulis* deployment and retrieval. *Mytilus edulis* were deployed at CNTR, 400E, 1000E, and REFS at the CLIS disposal site (Figure 2). The physical arrangement at each station is detailed by Phelps and Galloway (1980). In short, each station consisted of a surface buoy attached by cable to a concrete mooring on the bottom, with two smaller satellite moorings attached to the larger main mooring. A subsurface buoy was attached to each small mooring from which the mussel baskets were hung 1 m above the bottom. Two baskets were attached to each subsurface buoy at each deployment.

51. Deployment of *M. edulis* at the CLIS disposal site is summarized in Table 3. Mussels were deployed at each station for a period of 1 month predisposal to collect baseline data (cruise number T - 4). A second deployment occurred during disposal operations, except that no mussels were placed at the

Table 3

Cruise Number, Deployment Date, Retrieval Date, and Length of Deployment for Mussels Transplanted to CLIS

Cruise Number weeks	Deployment Date	Retrieval Date	Length of Deployment
T - 4	16 Mar 83	22 Apr 83	1 month
T = 0*	22 Apr 83	24 May 83	1 month
T + 2	23 Apr 83	07 Jun 83	6 weeks
T + 8	07 Jun 83	13 Jul 83	1 month
T + 12	13 Jul 83	10 Aug 83	1 month
T + 15	10 Aug 83	06 Sep 83	1 month
T + 21	16 Mar 83	18 Oct 83	7 months
T + 27	06 Sep 83	29 Nov 83	3 months
T + 43	29 Nov 83	20 Mar 84	3 months
T + 55	18 Oct 83	05 Jun 84	8 months
T + 74	12 Jun 84	17 Oct 84	4 months

* T = 0 refers to the termination of disposal activities at the FVP site on 18 May 1983.

CNTR station (T = 0, T + 2). Mussels were deployed for 1-month periods over the next 3 months (T + 8, T + 12, T + 16) and then on a quarterly basis for the next year (T + 23, T + 40, T + 55, and T + 74). In addition, several sets of mussels were left at each station for 7 months (T + 22).

52. *Mytilus edulis* were retrieved from the subsurface buoys by divers. Mussels used for chemical analysis were frozen immediately. The remaining mussels were maintained in tanks of flowing seawater on deck and returned to ERLN later that day and held in flowing unfiltered seawater overnight. The next morning, mussels were distributed to the appropriate investigators for biological analyses.

53. *Mytilus edulis* field exposures via tissue residues. Exposure conditions present in the field during each mussel deployment were not as well characterized as they were in the laboratory studies. As a result, the description of *M. edulis* exposure to BRH material in the field is more qualitative than quantitative and will be presented in two parts. First, a

prediction of field exposure is based on mussel tissue residues. The relationship between exposure to BRH sediments and tissue residues was determined in the laboratory experiments. Tissue residues from the 0-, 10-, and 30-percent BRH treatments at 28 days were regressed against measured BRH exposure concentrations (0, 1.5, 3.3 mg/ℓ) from the same exposures. In order to correct for background residues in the laboratory, the PCB concentration of the 0-percent BRH treatment was subtracted from the others prior to regression analysis. The resultant equation, mg/ℓ BRH material = (PCB residue × 0.000965) - 0.0019, (R^2 = 0.99) , was then used to calculate the average sustained concentration of BRH material necessary to achieve the residue value obtained in the field. The estimated BRH exposures in the field were determined by substituting the mussel PCB tissue residue concentration directly into the above equation. This estimate was assumed to represent an upper range of suspended BRH material present. A second estimate was determined by first subtracting the PCB concentration in mussels at the REFS station from the other stations during that collection. This removed the Long Island Sound background PCB levels from the estimates and thus was assumed to represent a lower range of BRH present in CLIS. This procedure was completed for each collection date and station that mussels were retrieved.

54. *Mytilus edulis* estimated exposure via water chemistry data. A second estimate of exposure was generated from the PCB and copper concentrations in the whole water samples collected during the various postdisposal cruises. The concentration of BRH material that would have to be present to produce these levels was determined by dividing the concentration of PCB and copper present in the barrel material collected from BRH (2,900 µg/g and 6,910 µg/g for copper and PCB, respectively). A range of exposures also was calculated for the water chemistry data; estimated BRH material was determined with and without subtracting the concentration at the REFS station.

55. *Nephtys incisa* field exposures via tissue residues. The purpose of exposure assessment is to determine the temporal and spatial range of exposure concentrations experienced by populations of interest. The exposure conditions present in the field for *N. incisa* were not as well characterized as they were in the laboratory studies. As a result, the description of *N. incisa* exposure to BRH material in the field is more qualitative than quantitative and is presented in three parts. First, a prediction of field exposure can be made based on worm tissue residues. The relationship between

exposure to BRH sediments and tissue residues was determined in the laboratory experiment. Tissue residues of PCBs as Aroclor 1254 (A1254) from the 0-, 50-, and 100-percent BRH treatments at 42 days were plotted against BRH exposure concentrations. This relationship was used to estimate field exposure conditions based on tissue residues of PCBs in field-collected worms. Inherent in this approach is the assumption that organisms have comparable patterns of bioaccumulation in the laboratory and in the field.

56. *Nephtys incisa* field exposures from physical data. A second analysis calculates the maximum total suspended solids concentrations from 1 m above the bottom to the sediment-water interface. This analysis assumes that the total suspended solids are composed totally of BRH sediments and represents a worst case or upper bound prediction. A third approach calculates the probable amount of BRH sediment exposure at the sediment-water interface based upon actual sediment contaminant concentrations for each sampling station and date. This analysis assumes that resuspension of the surface sediment is the primary source of the total suspended solids at the sediment-water interface.

57. The equation used to calculate total suspended solids concentrations from the sediment-water interface up to 1 m above the bottom is described as follows:

$$C_z = C_m\left[1 + (C_o - 1)\ e^{-kz}\right] \tag{4}$$

where

C_z = total suspended solids concentration at distance z

C_m = total suspended solids concentration at 1 m above the bottom

C_o = enrichment factor (C_z/C_m when $z = 0$)

$-k$ = rate of change in total suspended solids concentration as a function of z

z = distance from the bottom, m

Given the total suspended solids concentration at 1 m above the bottom, the equation predicts an exponential increase in suspended solids concentration at distances from 1 m above the bottom to the sediment-water interface.

58. The total suspended solids concentrations for these analyses were selected to represent average and storm conditions empirically determined from an in situ continuous monitoring platform deployed 1 m above the bottom at the disposal site (Bohlen and Winnick 1986; Munns et al. 1986). Enrichment

factors were likewise empirically determined from acoustic profilometer data collected between the sediment-water interface and 1 m above the bottom (Bohlen and Winnick 1986; Munns et al. 1986).

Maximum upper bound estimate

59. For the purposes of the maximum upper bound analyses, it was assumed that the exposed populations are located off the mound and aligned with the mean direction of current flow. The route of contaminant exposure was assumed to be through the transport of resuspended BRH sediments. These total suspended solids are composed of resuspended Long Island Sound sediments, as well as BRH sediment resuspended from the disposal site. Since the intent of these analyses is to create a maximum upper bound set of exposure conditions, it was assumed that the suspended solids concentration was composed, in total (100 percent), of resuspended BRH sediment.

Probable exposure estimate

60. It was not within the scope of this program to provide a continuous temporal record of the percent contribution of BRH sediments to the total suspended solids load. Consequently, a second set of analyses was designed to estimate the percentage of BRH sediment that could have comprised the total suspended solids concentration at the sediment-water interface for each station and indicate how these concentrations changed with time throughout the study. The proportions of BRH dredged material in the surficial sediments at each station and date were estimated by comparing the concentrations of selected contaminants measured in the 0- to 2-cm layer of sediment cores collected, postdisposal, at the FVP site. These field concentrations were compared with the barrel concentrations to determine a percentage as follows:

$$\text{Percentage BRH Sediment} = \left[\frac{(C - REF)}{(BRH - REF)}\right] \times 100 \quad (5)$$

where

C = concentration of contaminant in the sediment core

REF = concentration of contaminant in REF sediment

BRH = concentration of contaminant in BRH sediment (barrel)

The percentage BRH sediment values were calculated for each station and date using the 11 different contaminants, the details of which are shown in Appendix A, Tables A1-A13. To achieve a BRH-suspended sediment concentration that reflects the surficial sediment contaminant levels for each station and date,

the total suspended solids concentrations predicted for the sediment-water interface were multiplied by the estimated proportions of BRH sediment.

Chemical Methods

Analytical methods

61. The analytical methods used in this study are presented here in summary form. More detailed descriptions of the analytical methods are available in Lake, Hoffman, and Schimmel (1985). Most of these methods represent extensive modifications of USEPA standard methods developed for freshwater and wastewater samples. It was necessary to modify these methods in order to analyze the types of matrices in this study. These methods were intercalibrated to ensure the quality of the data.

Organic sample preparation

62. Samples of sediment, suspended particulates, and organisms were extracted by multiple additions of increasingly less polar organic solvents using a tissue homogenizer. These mixtures were separated by centrifugation between additions; polar solvents were removed by partitioning against water; and the extracts were desulfured with activated copper powder when required. The extracts were then passed through a precolumn containing activated silica gel. Samples of both filtered and unfiltered seawater were solvent extracted in separatory funnels, and the extracts were saved. Foam plugs containing the dissolved organic contaminants from water samples were extracted with organic solvents. All of the above extracts were subjected to column chromatography on deactivated silica gel to separate analytical fractions and were volume reduced carefully prior to analysis.

Organic analysis

63. Electron capture gas chromatographic analyses for PCBs were conducted on a Hewlett-Packard 5840 gas chromatograph equipped with a 30-m DB-5 fused silica column. Samples were quantified against an A1254 standard because the distribution of PCB congeners in the dredged material closely matched that distribution, as did the distribution in organisms at steady-state.

64. Gas chromatograph/mass spectrometric analyses were conducted with a Finnigan Model 4500, also equipped with a 30-m DB-5 fused silica capillary column. The mass spectrometer was operated through a standard Incos data

system and was tuned at all times to meet USEPA quality assurance specifications.

65. All instruments were calibrated daily with appropriate standards. The concentrations of the standards used were chosen to approximate those of the contaminants of interest, and periodic linearity checks were made to ensure the proper performance of each system. When standards were not available, response factors were calculated using mean responses of comparable standards. Blanks were carried through the procedure with each set of samples, and reference tissue homogenate was analyzed with every 12 to 15 tissue samples.

Organic data reduction

66. As stated above, PCBs were quantified as A1254 because the sample patterns closely resembled that profile. This allowed a convenient way of reporting these data without treating the voluminous data that would have resulted from measuring some 55 congener peaks by electron capture detector. Likewise, a method was sought to summarize the PAH data. Appendix B lists the 35 individual PAH parent and alkyl homolog compounds and groups of compounds measured in this study. Each PAH of the same molecular weight, both parents and alkyl homologs, can be summed to yield 9 PAH parent sums and 5 alkyl homolog sums. Although useful, this only reduced the data to 14 PAH variables, which was not sufficient. Since the distribution of PAHs differed greatly in both quantity and quality between Long Island Sound and the BRH dredged material, statistics were sought that would retain significant quantitative and qualitative information. The quantitative statistic chosen was the simple SUM of all measured PAHs, and a qualitative descriptor was chosen by analogy with the center of mass concept from elementary physics and called a centroid (CENT):

$$SUM = S[C(i)] \tag{6}$$

$$CENT = \frac{S[C(i) * MW(i)]}{SUM} \tag{7}$$

where

S = SUM

C(i) = concentration of i^{th} PAH from molecular weight 166 through 302, including both parent and alkyl homologs

MW(i) = molecular weight of i^{th} PAH from 166 through 302, including both parent and alkyl homologs

In this case, CENT describes the "center of mass" of the PAH distribution and is in units of molecular weight. It is the concentration-weighted average molecular weight of any particular PAH distribution. This statistic can be used to readily distinguish two different sources of PAH distributions; one with predominately heavy molecular weight pyrogenic compounds and one with more lighter molecular weight petrogenic compounds. These distributions are typically found in Long Island Sound at REFS and BRH, respectively. A major value to this statistic is that it enables one to readily distinguish these two sources when their contaminant concentrations are nearly equal. The formulas for calculating these, and 178 alkyl homologs, are shown in Appendix B. Because distributions of both parents and homologs were measured, SUMs and CENTs of both parents and homologs were calculated as well. These were defined as PSUM, PCENT, HSUM, and HCENT. By definition,

$$SUM = PSUM + HSUM \tag{8}$$

and

$$CENT = \frac{PSUM * PCENT + HSUM * HCENT}{SUM} \tag{9}$$

It should be noted that dibenzothiophene and its alkyl homologs are not included in these calculations because they are not PAHs.

Inorganic sample preparation

67. Sediment was prepared for inorganic analysis by elution at room temperature with 2N HNO_3. The samples were filtered through Whatman #2 filter paper. Organisms were totally digested in concentrated HNO_3 at 60° C and filtered through Whatman #2 filter paper.

68. Cadmium, nickel, lead, and copper were concentrated and separated from both the unfiltered and filtered seawater fractions by coprecipitation (Boyle and Edmond 1975). The remaining metals (chromium, iron, manganese, and zinc) were analyzed by heated graphite atomization atomic absorption (HGA-AA) via direct injection. Samples of suspended particulates on Nucleopore (0.45 μ) filters were eluted with 2N HNO_3 and analyzed by HGA-AA.

Inorganic analysis

69. All flame atomization atomic absorption (FA-AA) was conducted with a Perkin-Elmer (Model 5000) atomic absorption spectrophotometer. All HGA-AA

determinations were conducted with Perkin-Elmer Model 500 or 2100 HGA units coupled to Perkin-Elmer Model 5000 or 603 atomic absorption instruments, respectively. The Model 5000 AA was retrofitted with a Zeeman HGA background correction unit, and the Model 603 was equipped with a D2 arc background correction system.

70. The FA-AA and HGA-AA instrument operating conditions are similar to those described in USEPA (1979) and those in the manufacturers' reference manuals. The AA instruments were calibrated each time samples were analyzed for a given element. Sample extracts were analyzed a minimum of twice to determine signal reproducibility. Quality assurance checks, conducted after every 15 samples, were analyzed by the method of standard addition and by analyzing one procedural blank.

Contaminant selection

71. Chemical analyses performed in this study characterize the organic and inorganic constituents in the dredged material, provide information on the laboratory and field exposure environments, provide insight into the processes governing contaminant movement within and between environmental compartments, and determine which contaminants were accumulated by organisms. In determining the acceptability of dredged material for ocean disposal, a variety of evaluatory criteria are applied. These include bulk sediment chemistry, toxicity, and bioaccumulation. In this study, bioavailability was determined by examining the types and distributions of contaminants that bioaccumulated in laboratory studies (Rogerson, Schimmel, and Hoffman 1985). Based upon the contaminant profile for the dredged material and residue data, the contaminants selected for detailed analyses throughout the study included PCBs, PAHs, the pesticide ethylan, and eight metals.

72. A representative subset of chemicals was selected for discussion throughout the study. The criteria used in selecting this subset included chemical properties, contaminant representativeness and behavior in various compartments, and statistical analyses of the distributions of the complete suite of chemicals analyzed in the program.

73. Multivariate clustering analyses were performed on the chemical data in an attempt to define groups or clusters of chemicals that behaved in a statistically similar manner. No assumptions were made concerning the behavior, interactions, or dynamics of chemicals between compartments; therefore, each compartment was analyzed separately. Five compartments were identified

from field and laboratory data for statistical analysis. Of these, the surficial sediments and the unfiltered, particulate, and dissolved water column fractions described exposure conditions experienced by infaunal and pelagic organisms. The remaining compartment consisted of tissue residues in organisms.

74. The data were further partitioned into inorganic and organic analysis. The inorganic analyses generally consisted of 8 variables, whereas the organic analyses contained 61 variables. The clusters of chemicals identified through the statistical analyses agreed well with those contaminants selected based on chemical properties and environmental behavior. The subset of chemicals selected as representative included six organic compounds, four metals, and two summary statistics.

Statistical Analysis Methods

75. The primary objective of the FVP was to compare laboratory with field responses under similar exposure conditions. Because of the highly dynamic temporal and spatial conditions in the field, the exposure environment can be given only boundaries and cannot be assigned specific values, as is the case for laboratory studies. Consequently, the degree to which laboratory exposure-response relationships concur with those derived from field data can be described only qualitatively. That does not preclude the use of inferential statistical procedures to explore those laboratory and field relationships for which the appropriate quantitative information is available. However, the nature of this project was such that descriptive and exploratory statistics were often the most appropriate techniques to illustrate relations and trends. Simple graphic representations of variables were all that was necessary to illustrate a relationship. In addition, multivariate techniques, such as cluster analysis, were the most appropriate techniques to elucidate more complex relationships between groups of selected variables.

76. Prior to making comparisons between laboratory and field effects, it was necessary to establish whether field exposure boundaries were similar to those measured in the laboratory. Assuming that tissue residue and exposure are closely related, this was accomplished by examining the tissue residues of all worms from laboratory and field exposures together, independent of exposure concentration or station location and date. An agglomerative

hierarchical cluster analysis was performed on the ten selected chemical contaminants and the two summary statistics using the SAS cluster procedure (SAS 1985) to establish which tissue residues among all the laboratory treatments and field stations were most similar. The clustering procedure used was the average linkage method, which uses unweighted pair-groups with arithmetic averages on squared distances between samples. Prior to analysis, residue data were normalized using standard deviations from the mean. This procedure ensured that each variable was weighted equally, even if its absolute value was orders of magnitude different from another variable.

77. The relationship between AEC and tissue residue values in the field samples was explored by regressing and plotting the mean AEC value for each sample against the corresponding mean tissue residue (Snedecor and Cochran 1980). This procedure was completed individually for each of the 10 selected chemical contaminants and the two summary statistics.

PART III: RESULTS

Laboratory

Exposure

78. *Mytilus edulis* system monitoring. The *M. edulis* exposure system was monitored for both total suspended solids (TSS) concentrations and the percentage of REF and BRH sediments. A strip chart record indicated that the system maintained a suspended particulate concentration of 10 mg/ℓ approximately 90 percent of the time. Examples of times when the 10 mg/ℓ was not maintained include periods when exposure tanks were cleaned, slurry reservoirs were changed, and lines were clogged. Overall, the system provided a nearly constant total suspended particulate concentration to the mussels. The concentration of BRH sediments dosed into each treatment is listed in Table 4.

Table 4

Suspended Sediment Concentrations in the Mussel Exposure System

Nominal % BRH	Measured % BRH	Calculated BRH Sediment mg/ℓ
100	100(0.0)*	10.0
50	50(0.83)	5.0
30	33(0.84)	3.3
10	15(1.39)	1.5
0	0(0.0)	0.0

* Standard error in parentheses.

79. When the TSS concentration dropped in the 50-percent BRH exposure tank, a pulse of equal length was sent to both the REF and BRH dosing valves. Volumetric measurements of the BRH and REF sediment doses indicated that equal amounts (±5 percent) of BRH and REF material were delivered to the 50-percent BRH exposure chamber. The 100- and 0-percent BRH treatments were controlled by single dosing values.

80. The 10- and 30-percent BRH treatments required two dosing valves per treatment. Because the pulse length could not be adjusted separately for each valve, manual adjustment of each valve was required to provide the desired concentration. The volumetric amount of BRH and REF material delivered

to each treatment was monitored and recorded. In the treatment with a nominal 10-percent BRH, the actual value delivered was 15 percent. In the 30-percent BRH treatment, the actual value was 33 percent.

81. *Mytilus edulis* chemical monitoring. The results of the chemical monitoring are prefaced by a brief restatement of the purpose of the exposure system to aid in the understanding of the results. The system used in this experiment was designed to maintain a constant particulate concentration of 10 mg/ℓ in the exposure chambers. Initially, 150 animals were placed into each chamber with clearance rates of approximately 2 ℓ/mussel/hr, or a total of 300 ℓ/hr. The seawater flow rate through each chamber, independent of suspended sediment additions, was approximately 24 ℓ/hr. In effect, suspended sediment was added at a rate 12.5 times that of seawater to each exposure chamber each hour to compensate for sediment removed by the mussels. This has important consequences on the behavior of the contaminants in the exposure system.

82. If all the contaminants were associated with the suspended sediment, the contaminant concentrations in the exposure chambers should be similar to those predicted by regressing the TSS concentrations with contaminant concentrations in the BRH material. Conversely, any contaminants that do not remain bound to the particulates could attain concentrations in the exposure system different from those predicted from the TSS data. This occurs because the mussels in the system are more efficient at removing the particulate-bound contaminants than they are at removing the dissolved contaminants. This theory is proposed to explain the measured chemical concentrations in the exposure system, using PCB and copper as examples.

83. Whole water samples were taken for chemical analysis on days 1, 7, 14, 21, and 28 in the second experiment. The mean PCB concentrations (nanograms per litre) for the five sampling dates for each exposure treatment in the second experiment are given in Table 5. The corresponding concentration of BRH sediment was estimated by regressing the nominal concentration of BRH against the expected value of PCB. Expected PCB concentrations were based on the PCB concentrations in the BRH sediment (6 ng/mg) plus background seawater concentrations. Substitution of the actual measured values of PCBs in the exposure system into the equation provided an estimated value of the concentration of BRH sediment in the system. The estimated concentration of BRH sediment in each treatment is similar to the actual measured values. These data

Table 5

Chemical Monitoring of the Exposure System in Experiment 2

Nominal Treatment Concentration % BRH	PCB Concentration, ng/ℓ		BRH Concentration, mg/ℓ	
	Expected	Measured	Estimated	Measured
0	1.1	2.2	0.2	0
10	7.1	11.9	1.8	1.5
30	18.8	23.6	3.8	3.3

suggest that PCB concentrations in the system are closely associated with the TSS concentrations.

84. Copper concentrations were measured both with and without mussels in the exposure system at 10 mg/ℓ TSS for each treatment. With no mussels in the exposure system, the total copper concentrations were 9.37 and 2.5 µg/ℓ for the 30- and 10-percent BRH treatments, respectively. These concentrations represent 3.8 and 1.8 mg/ℓ BRH sediment in the two treatments, respectively. Under these conditions, the predicted and measured copper concentrations were comparable. This resulted because the effective flow of suspended sediment and incoming seawater is the same. The only loss of TSS was out the overflow due to seawater flow rates.

85. When mussels were present in the system, the mean copper concentrations were 17.0 and 10.7 µg/ℓ for the 30- and 10-percent BRH treatments, respectively. These copper concentrations correspond to 68- and 43-percent BRH sediment in the two treatments, respectively, and conflict with those expected from the TSS data. The results may be explained by the fact that copper, due to its solubility in seawater, became disassociated from the TSS. Because suspended solids were delivered at a higher rate to the exposure chamber than the rate of incoming seawater, soluble copper accumulated in the exposure chamber. When a dose of BRH suspended sediment was delivered to an exposure chamber, all contaminants were introduced at the same rate. Because the mussels were more efficient at removing particulates than dissolved contaminants, dissolved copper tended to accumulate because its removal from the system was primarily via the overflow at a much slower rate. This resulted in higher concentrations of copper than those predicted from the TSS data alone.

86. *Nephtys incisa* system monitoring. During the three *N. incisa* laboratory experiments, the exposure system was monitored for TSS, seawater

temperature, and seawater salinity. These data are presented in Table 6. In general, the exposure system maintained the suspended solids concentrations close to the nominal 200 mg/ℓ. Temperature and salinity values were stable at approximately 20° C and 30 g/kg, respectively. DO concentrations were checked once during each experiment, and they never differed significantly from expected saturation.

Table 6

Measured TSS Concentrations (Dry Weight) and Exposure Conditions for Laboratory Tests with *N. incisa*

Treatment % BRH	TSS, mg/ℓ $\bar{x}$ ± SD	Seawater Temperature $\bar{x}$ ± SD	Seawater Salinity, g/kg $\bar{x}$ ± SD
	Test 1 - 10 Dec 1984 (10 days)		
100	215 ± 53	19.9 ± 0.30	31.5 ± 0.70
75	182 ± 14	19.9 ± 0.30	31.5 ± 0.70
50	180 ± 19	19.9 ± 0.30	31.5 ± 0.70
25	189 ± 22	19.9 ± 0.30	31.5 ± 0.70
0	190 ± 16	19.9 ± 0.30	31.5 ± 0.70
	Test 2 - 12 Mar 1985 (28 days)		
100	183 ± 24	18.9 ± 0.43	31.1 ± 0.86
50	185 ± 21	18.9 ± 0.43	31.1 ± 0.86
0	203 ± 24	18.9 ± 0.43	31.1 ± 0.86
	Test 3 - 3 May 1985 (42 days)		
100	201 ± 23	19.8 ± 0.53	30.9 ± 0.70
50	184 ± 19	19.8 ± 0.53	30.9 ± 0.70
0	190 ± 21	19.8 ± 0.53	30.9 ± 0.70

87. *Nephtys incisa* chemical monitoring. During the 42-day experiment, seawater and *N. incisa* from the exposure chambers were sampled for chemical analysis. Seawater chemical monitoring data are presented in Table 7. The dosing system malfunctioned for 2 days spilling BRH sediments into all treatments. The day 18 chemistry samples were taken during this period. The problem was corrected, and the system performed normally for the remainder of the test. The seawater chemistry data confirm that *N. incisa* received a graded exposure to BRH sediments during most (40 of 42 days) of the experiment.

Table 7

Chemical Analysis of Seawater in Exposure Chambers of 42-Day Experiment Exposing *N. incisa* to BRH sediment

Experiment Day	Treatment % BRH	Total PCB ng/ℓ as A1254	Total Metals, µg/ℓ Cu	Cd	Cr
3	100	NS*	407	5.4	245
	50	NS	256	3.2	159
	0	NS	15	0.1	15
6	100	1,170	NS	NS	NS
	50	590	NS	NS	NS
	0	79	NS	NS	NS
18**	100	340	307	3.6	181
	50	510	208	3.5	125
	0	700	134	2.2	89
32	100	NS	357	5.0	203
	50	NS	171	2.6	106
	0	NS	15	0.1	16
42	100	1,920	NS	NS	NS
	50	980	NS	NS	NS
	0	12	NS	NS	NS

* Not sampled.

** Dosing system malfunctioned for 2 days spilling BRH sediments into all treatments.

Chemical analysis of test sediments

88. The contaminant-specific analysis of the BRH and REF sediments is presented in summary form for the representative subset of chemical compounds discussed in this report. These analyses demonstrate clearly the differences in contaminant concentration between the two sediments (Table 8).

Tissue residue

89. *Mytilus edulis.* Differences in contaminant concentrations between BRH and REF sediments facilitated the tracking of these contaminants in exposed biota. Results of Experiment 1 indicated that PCB tissue residue concentration in mussels are directly related to exposure concentration (Table 9). PCBs in mussels from the 0-percent BRH concentration remained about the same over the 14-day experiment.

90. The PCB tissue residue data from Experiment 2 are listed in

Table 8

Concentrations of the Ten Selected Contaminants and Two Summary Statistics for Both BRH and REF Sediments

Mean ± Standard Deviation

Chemical Compound	Sediment* BRH	REF
Phenanthrene	5,000 ± 1,800 (15)**	85 ± 17 (12)
Sum of 178 alkyl homologs	28,000 ± 8,300 (15)	170 ± 26 (12)
Fluoranthene	6,300 ± 1,300 (15)	240 ± 33 (12)
Benzo(a)pyrene	3,900 ± 970 (15)	250 ± 28 (12)
Ethylan	4,000 ± 820 (15)	0 ± - (12)
PCB as A1254	6,400 ± 840 (15)	39 ± 4 (12)
SUM of PAHs	142,000 ± 30,000 (15)	4,500 ± 510 (12)
CENT of PAHs	232.8 ± 1.7 (15)	249.2 ± 1.7 (12)
Copper	2,900 ± 310 (18)	60 ± 3 (15)
Cadmium	24 ± 1.0 (18)	0.23 ± 0.04 (15)
Chromium	1,480 ± 104 (18)	50 ± 15 (15)
Iron	31,000 ± 2,800 (18)	21,000 ± 1,400 (15)

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic CENT.

** (N) = number of replicates.

Table 10 and graphically depicted in Figure 11. Tissue residues, measured at 7-day intervals, indicated that the mussels in the 0-percent BRH chamber maintained a relatively constant background concentration of PCBs throughout the experiment. In the 10- and 30-percent BRH chambers, the concentration of PCBs in the mussels increased from days 0 to 14 and then remained nearly constant between days 14 and 28, suggesting that the mussels reached a steady-state somewhere between days 7 and 14. The steady-state PCB concentration in mussels in the 30-percent BRH treatment was almost double that of mussels from

Table 9

PCB Tissue Residues (ng/g Dry Weight) in Mussels from Laboratory Experiment 1

	% BRH		
Day	0	50	100
0	117	117	117
14	154	2,100	3,700

Table 10

PCB Tissue Residues (ng/g Dry Weight) in Mussels from Laboratory Experiment 2

	% BRH		
Day	0	10	30
0	210	210	210
7	280	1,110	2,100
14	270	1,910	3,600
21	360	1,720	3,600
28	280	1,840	3,700

the 10-percent BRH treatment. The actual concentration of BRH dosed to the 30-percent BRH treatment, 3.3 mg/ℓ, is nearly double that dosed to the 10-percent BRH treatment, 1.5 mg/ℓ. The measured whole water concentrations of PCB were 11.86 and 23.57 ng/ℓ for the 10- and 30-percent BRH treatments, respectively. These data indicate a good relationship between the actual dosed concentrations of BRH suspended sediment, the measured whole water concentrations, and the PCB tissue residues in the mussels in Experiment 2.

91. A comparison of the tissue residues between the two experiments can be made for days 0 and 14. The PCB residue concentrations in the day 0 mussels from Experiment 1 were almost half that of those from Experiment 2, (117 and 210 ng/g, respectively). In addition, day 14 PCB residue concentrations were about the same for the 10- and 50-percent BRH exposed mussels (1,910 and 2,100 ng/g) as well as the 30- and 100-percent BRH exposed mussels (3,600 and 3,700 ng/g). These data show dose responses within each experiment; however, there is poor agreement between experiments. PCB data from these experiments were normalized to nanograms per gram of lipid, and the results are presented

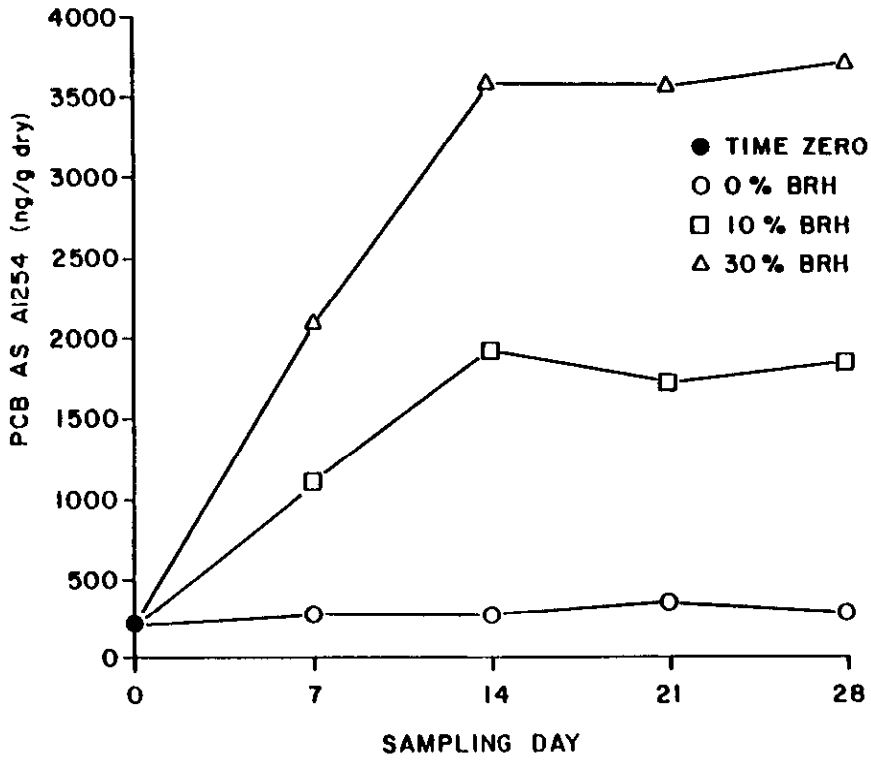


Figure 11. Concentrations of PCB as A1254 in the tissue of *M. edulis* exposed to BRH suspended sediments for 28 days

in Table 11 and Figure 12. Inspection of these data show that differences between experiments can be explained when differences in lipid content of the organisms are taken into account. In addition, this procedure indicates that a dose-response relationship does exist between experiments when the day 14 data from both experiments are combined (Figure 12).

Table 11

PCB Concentrations (ng/g Lipid) in Mussels from Both Laboratory Experiments

	% BRH Treatments					
Day	0*	0**	10**	30**	50*	100*
0	2,900	2,400	2,400	2,400	2,900	2,900
7	--	5,200	17,100	24,000	--	--
14	3,800	4,300	27,000	54,000	53,000	119,000
21	--	5,000	35,000	67,000	--	--
28	--	3,800	30,000	66,000	--	--

* Experiment 1.
** Experiment 2.

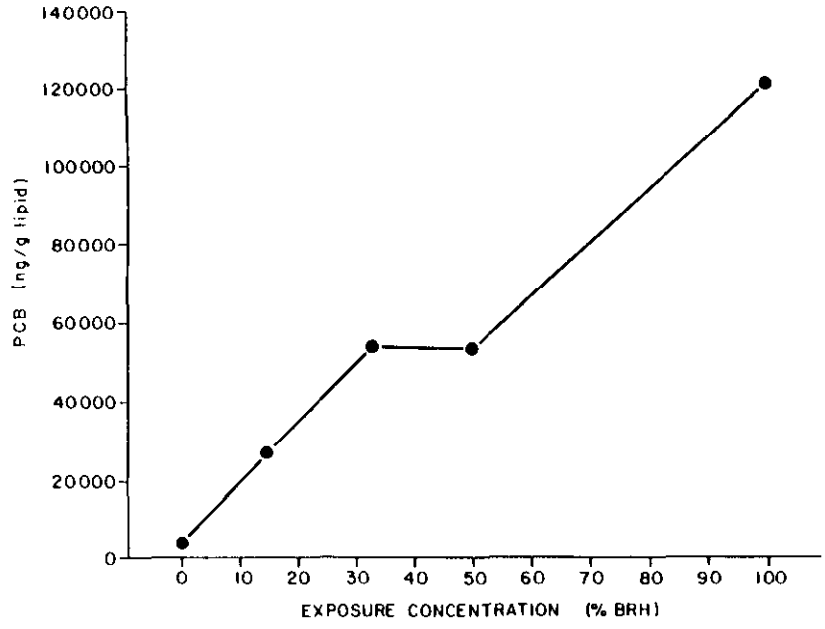
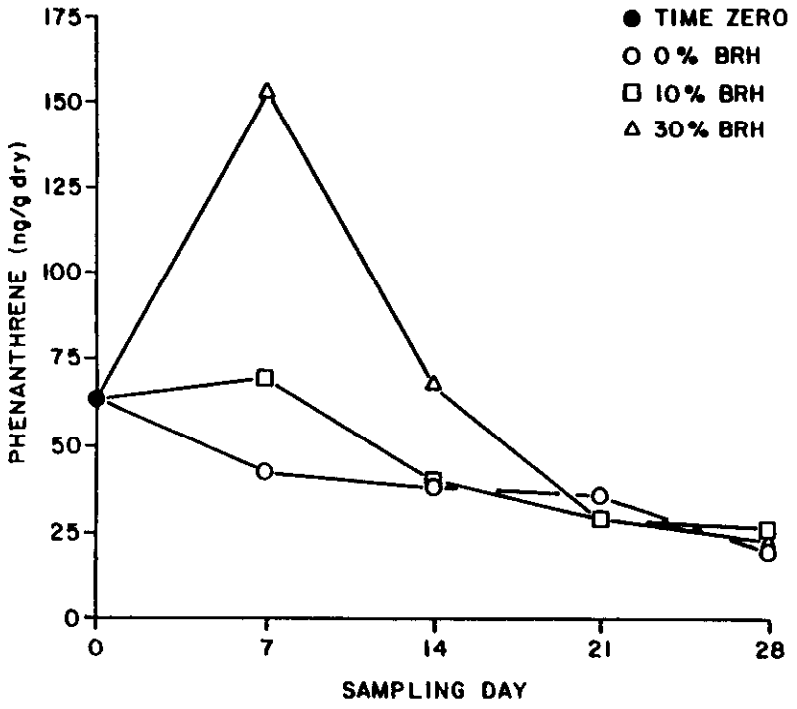


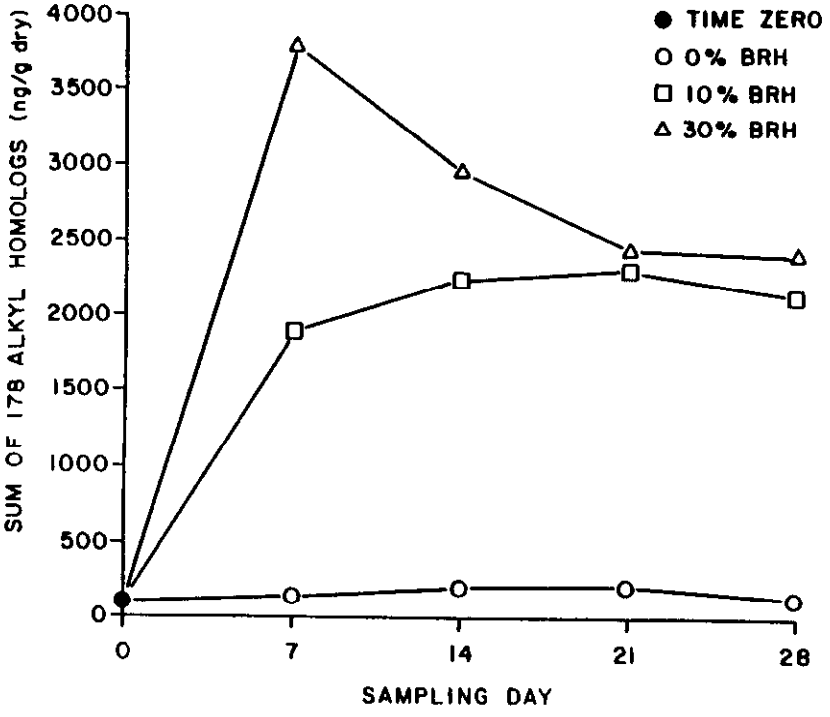
Figure 12. Concentrations of PCB as A1254, normalized for lipids, in the tissue of *M. edulis* exposed to BRH sediment for 14 days

92. In addition to PCBs, tissue residues of phenanthrene, the sum of the 178 alkyl homologs, fluoranthene, benzo(a)pyrene, ethylan, cadmium, copper, chromium, and iron were also measured on days 0, 7, 14, 21, and 28 of Experiment 2. The summary statistics, SUM and CENT, of the PAHs were also calculated for each of these sampling dates. These data are summarized graphically in Figures 13-18.

93. While each of these graphs will not be discussed at length, it is interesting to note the relationship between the molecular weight of the organic compounds and tissue residue over time. The benzo(a)pyrene tissue residues follow a pattern similar to that of PCB. After 7 days, residues remain nearly constant for each exposure concentration. The fluoranthene residues are initially higher in the 30-percent BRH treatment. However, they decrease over the 28-day exposure period to a level comparable with the 10-percent BRH treatment. Mussel residues for both of these treatments are elevated compared with the 0-percent BRH treatments. Phenanthrene, an even lower molecular weight PAH, increased initially but then decreased in both the 30- and 10-percent BRH treatments to a level comparable with the 0-percent BRH exposure. These data would suggest that mussels have the ability to metabolize and/or excrete the lower molecular weight PAHs, even during continuous

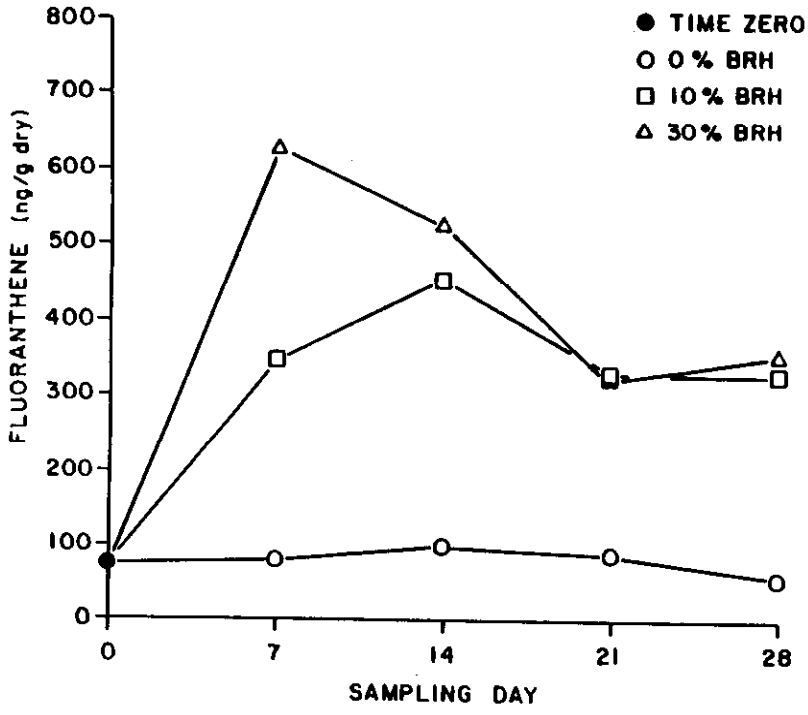


a. Phenanthrene

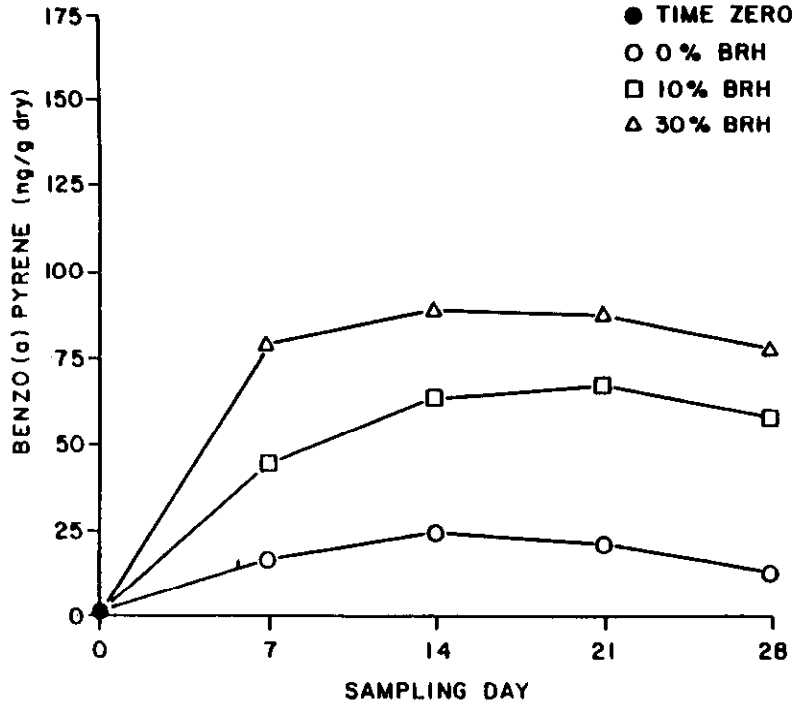


b. Sum of 178 alkyl homologs

Figure 13. Concentrations of phenanthrene and sum of 178 alkyl homologs in the tissue of *M. edulis* exposed to BRH suspended sediments for 28 days

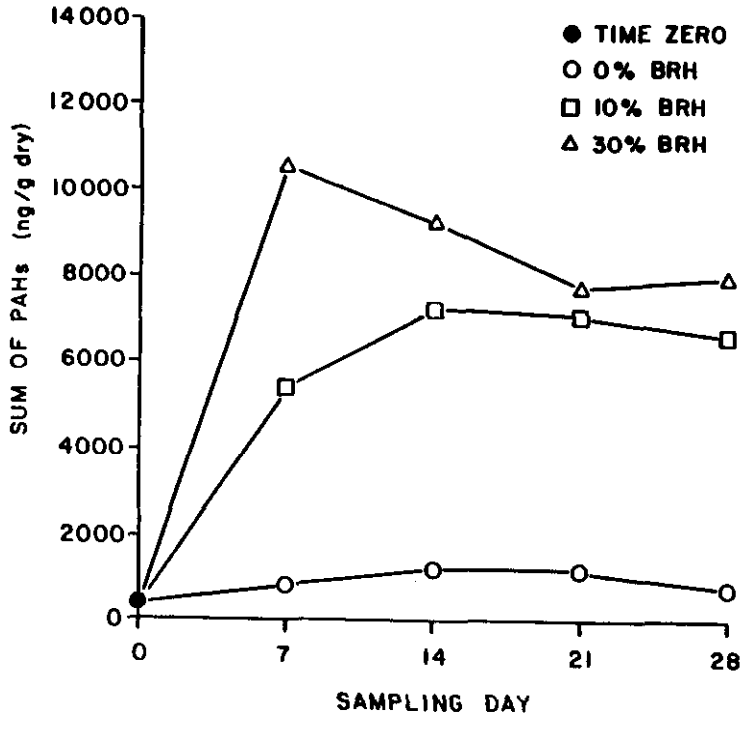


a. Fluoranthene

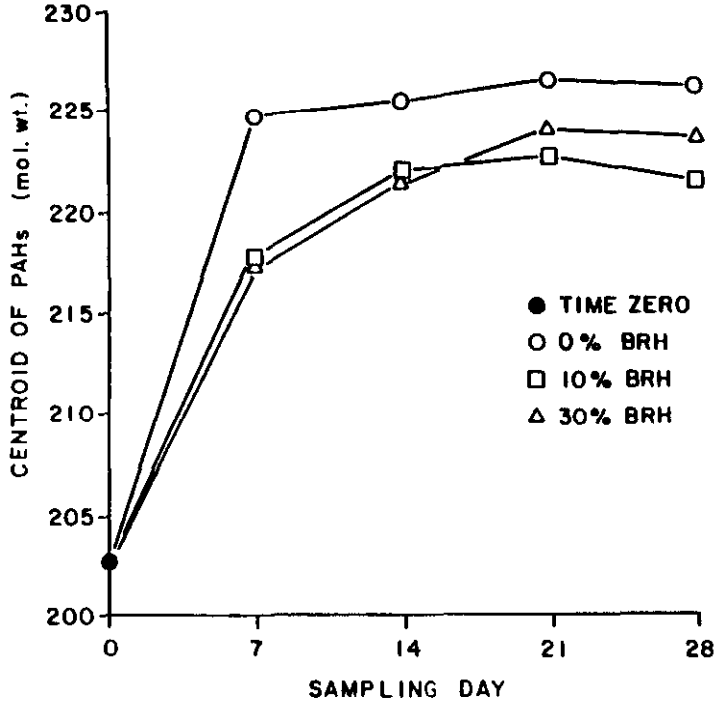


b. Benzo(a)pyrene

Figure 14. Concentrations of fluoranthene and benzo(a)pyrene in the tissue of *M. edulis* exposed to BRH suspended sediments for 28 days

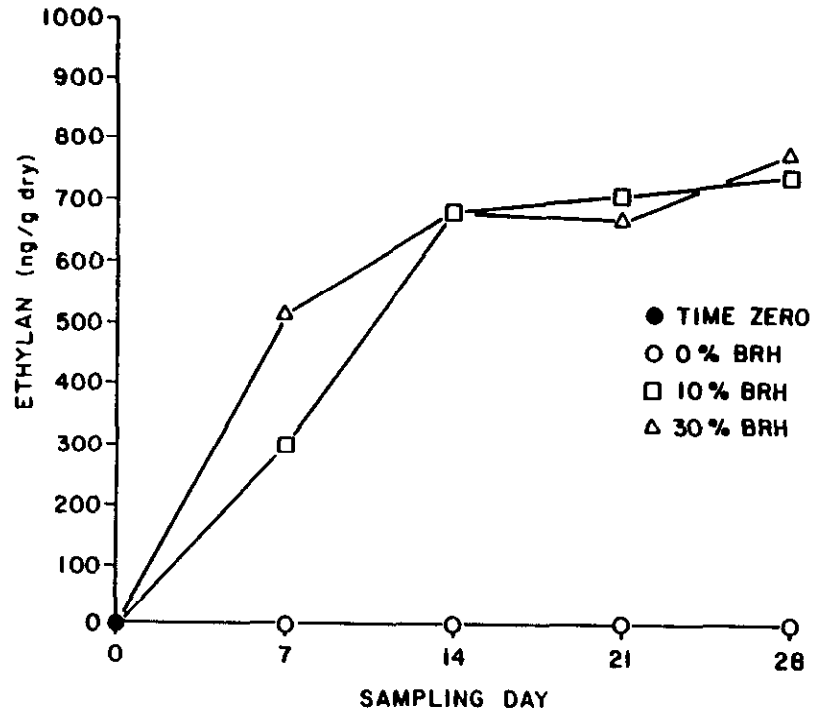


a. SUM of PAHs

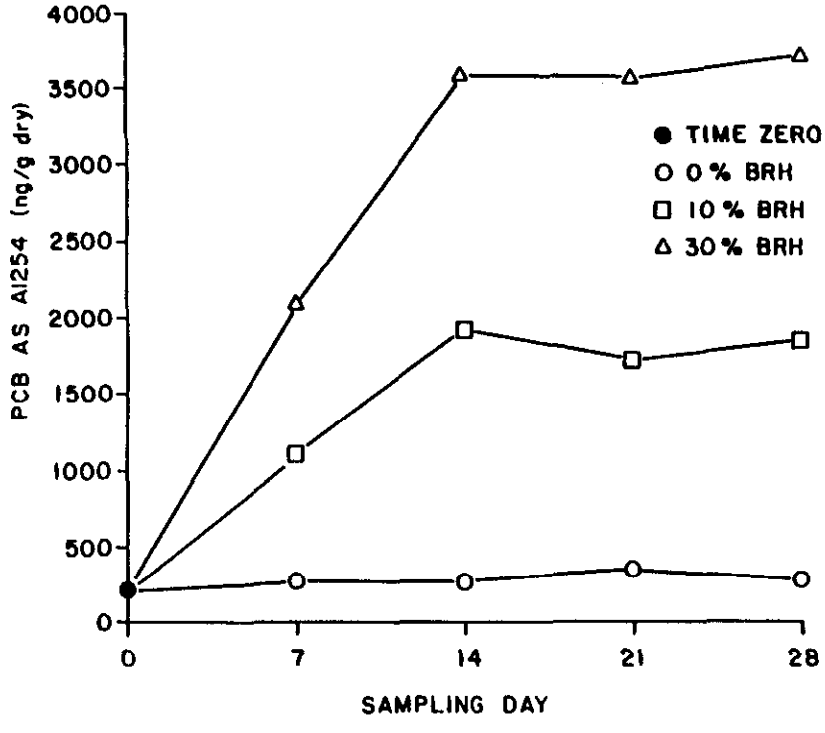


b. CENT of PAHs

Figure 15. Concentrations of SUM of PAHs and CENT of PAHs in the tissue of *M. edulis* exposed to BRH suspended sediments for 28 days

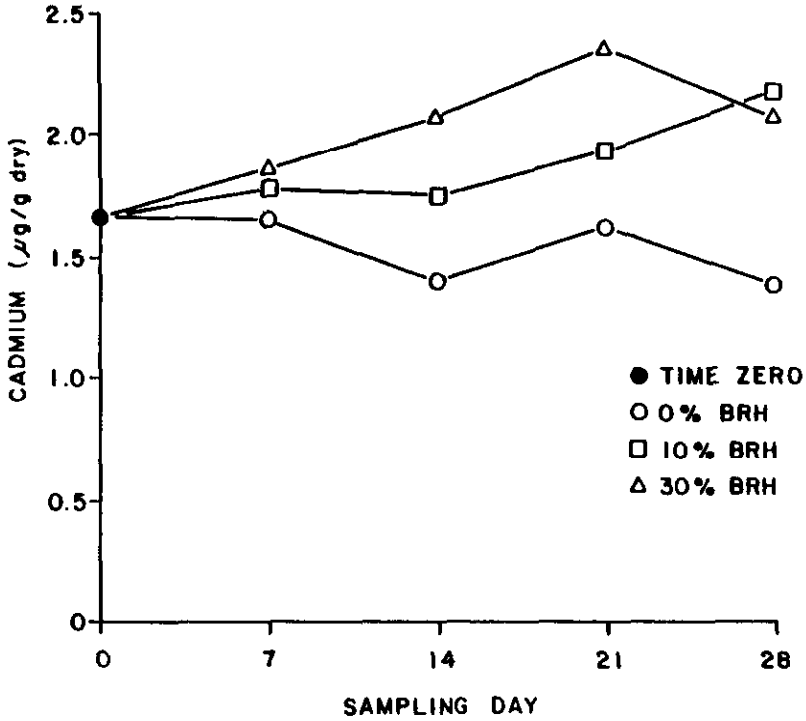


a. Ethylan

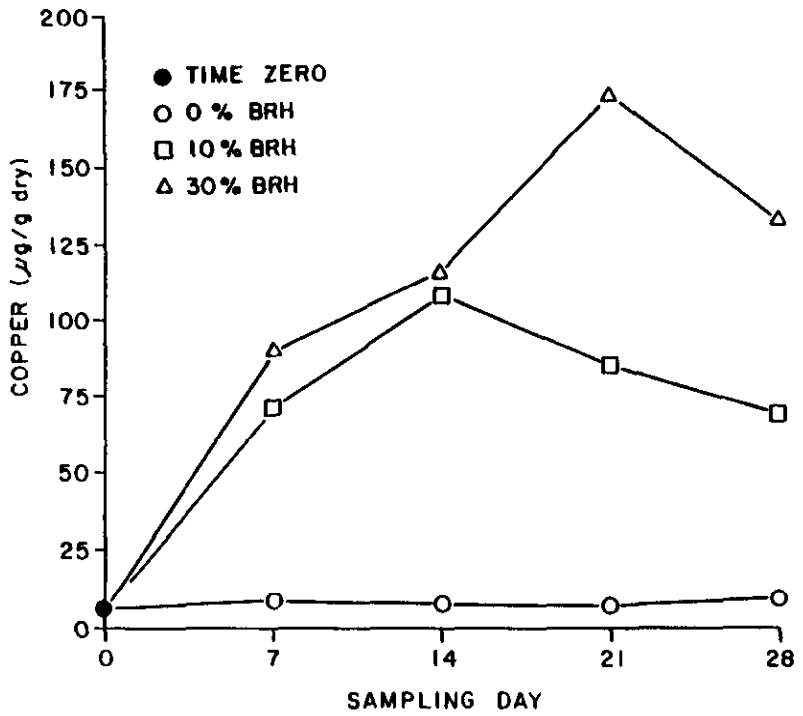


b. PCB as A1254

Figure 16. Concentrations of ethylan and PCB as A1254 in the tissue of *M. edulis* exposed to BRH suspended sediments for 28 days

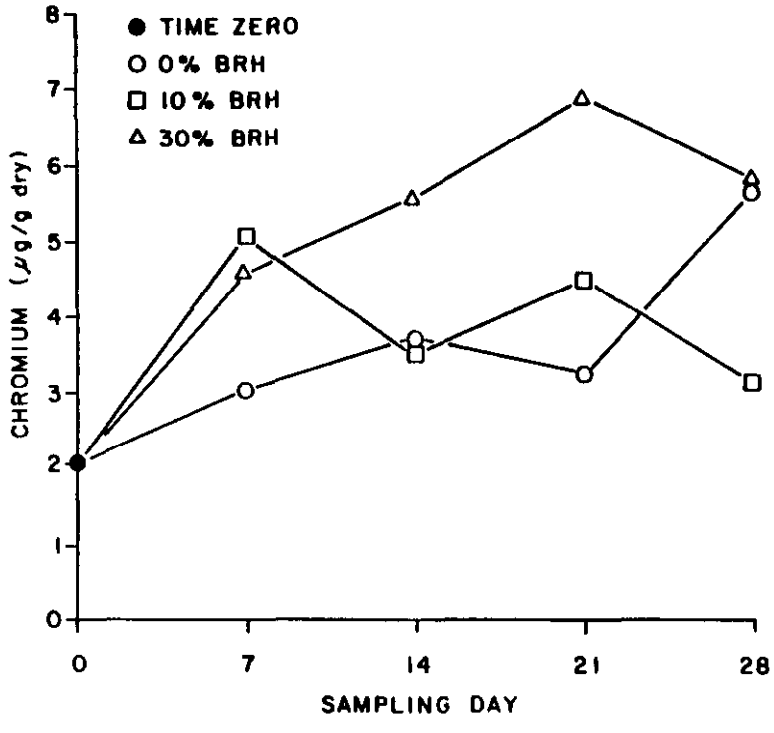


a. Cadmium

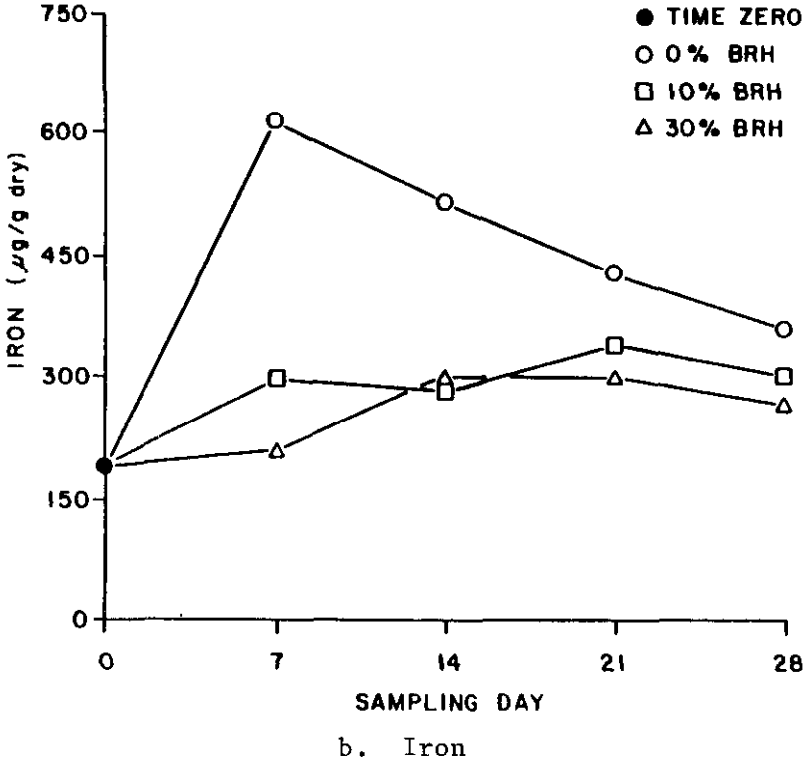


b. Copper

Figure 17. Concentrations of cadmium and copper in the tissue of *M. edulis* exposed to BRH suspended sediments for 28 days



a. Chromium



b. Iron

Figure 18. Concentrations of chromium and iron in the tissue of *M. edulis* exposed to BRH suspended sediments for 28 days

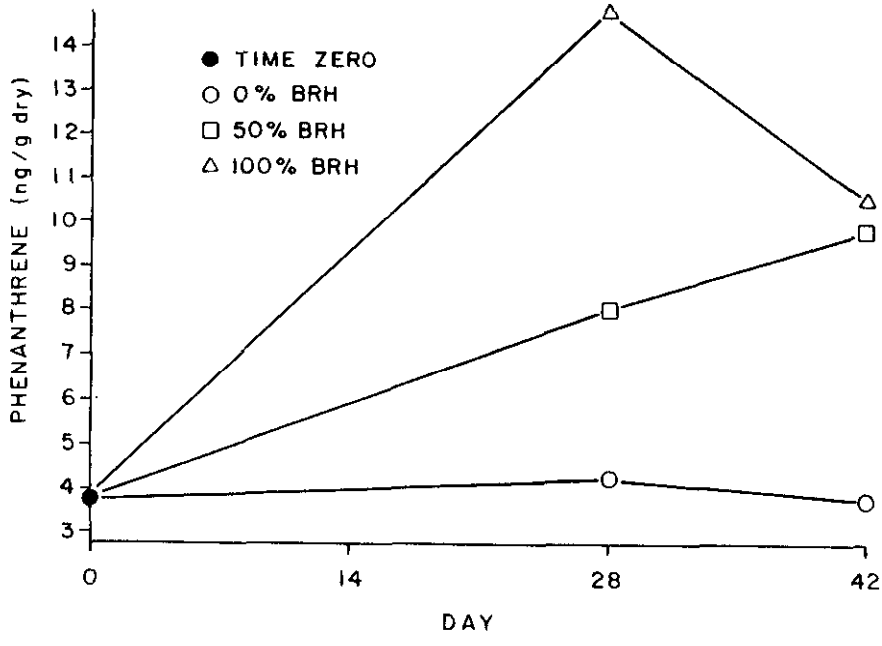
exposure. In addition, the data would indicate that only higher molecular weight compounds should be used to relate exposure levels and subsequent tissue residue levels, because even under relatively constant exposure conditions, residues of lower molecular weight PAHs did not reflect exposure concentrations.

94. *Nephtys incisa.* *Nephtys incisa* tissues from suspended sediment laboratory exposures were analyzed for a suite of organic and inorganic contaminants found in BRH sediment. These tissue residues were measured on samples from days 0, 28, and 42 of the experiment. The summary statistics, SUM and CENT, of the PAHs were also calculated for each of these sampling dates. The tissue residue data for the representative subset of chemical compounds are presented graphically in Figures 19-24.

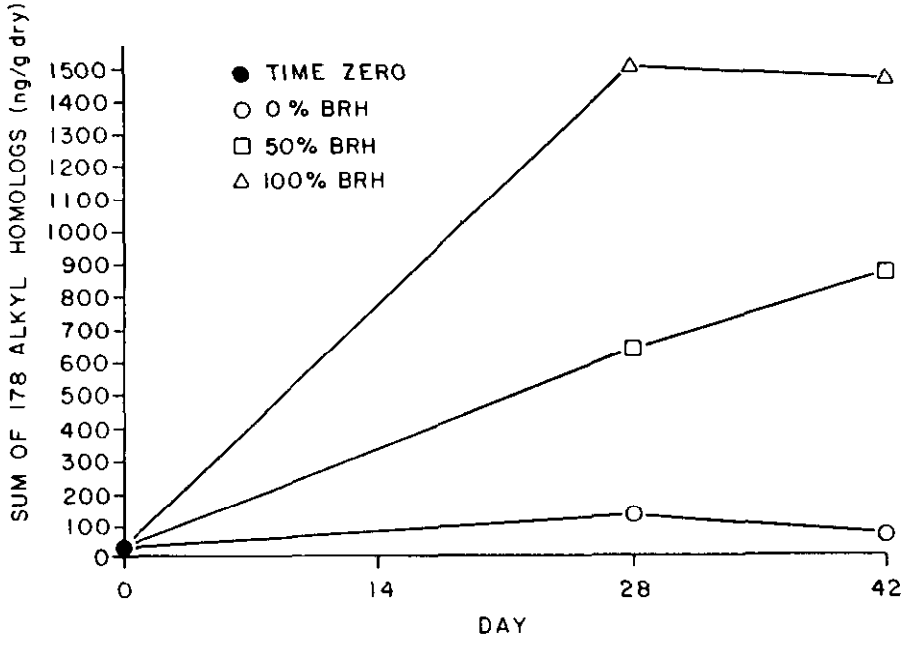
95. Although the data presented in these figures are not discussed in detail (see Lake et al. 1987), some general observations are made. The tissue residue concentrations of all the organic compounds increased with increasing exposure. The PAHs, with the exception of fluoranthene, reached their highest measured tissue concentrations at day 28 and exposure concentrations of 100-percent BRH (200-mg BRH/ℓ). By day 42, the residue concentrations of phenanthrene and benzo(a)pyrene declined by 30 and 50 percent, respectively. The tissue residue concentrations of PCBs reached an apparent steady-state at the 50-percent BRH (100 mg/BRH/ℓ) exposure by day 28, although there was a continued increase at 100-percent BRH (200 mg/BRH/ℓ) at day 42. Because of its kinetic, partitioning, and persistence properties, PCB was selected as a "tracer" for BRH material and was used to relate BRH exposure conditions to tissue concentrations. Copper and cadmium, which have soluble fractions in seawater, did produce elevated tissue concentrations as a consequence of increased exposure to BRH suspended sediment. Chromium and iron, which are bound to particulates, did not produce elevated tissue concentrations and in fact showed apparent depuration of these compounds from day 28 to day 42 of the experiment.

Effects results

96. *Mytilus edulis.* Adenine nucleotide concentrations were measured in adductor muscle tissues of *M. edulis* exposed to BRH sediments in both laboratory experiments. The results of these measurements are presented in Table 12. These data are presented graphically in Figure 25. The data for day 14 from both experiments represent an exposure range of 0 to 10 mg BRH/ℓ.

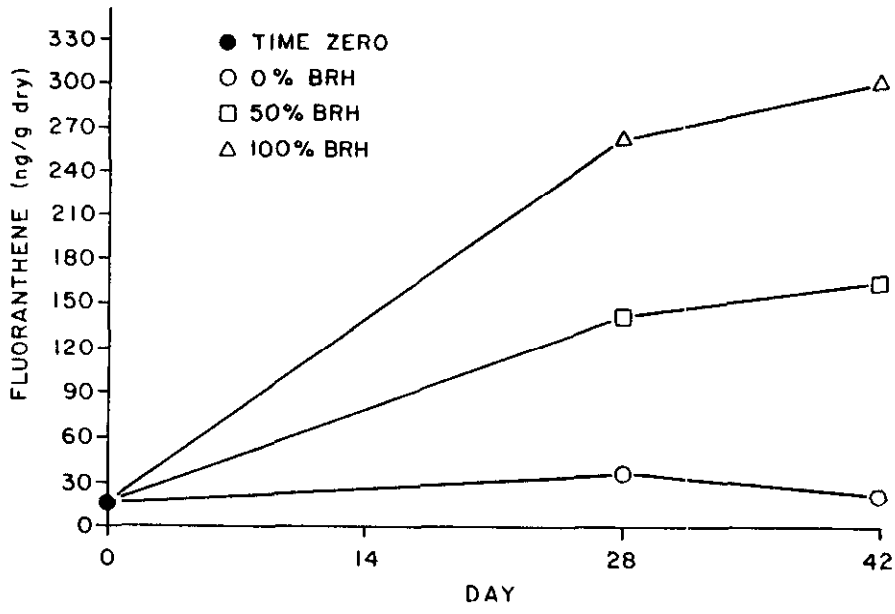


a. Phenanthrene

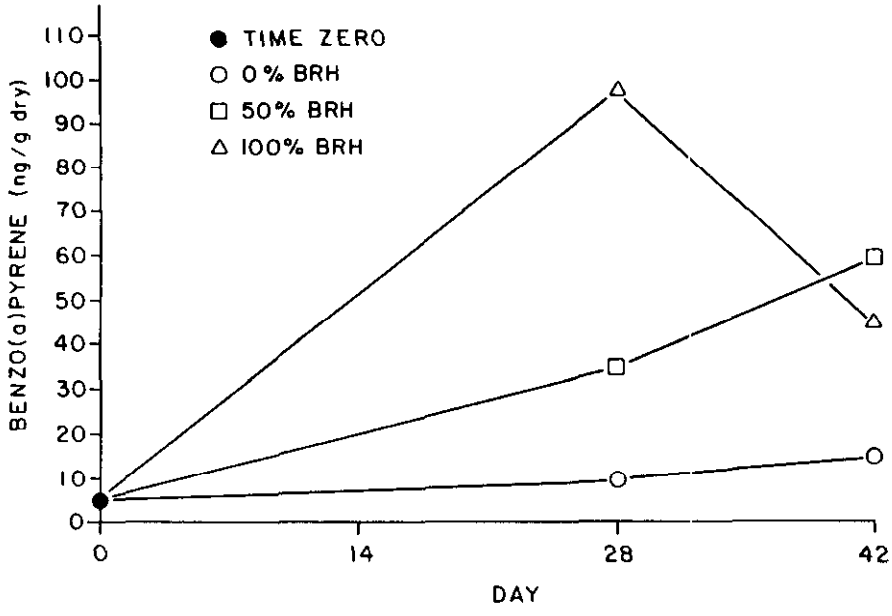


b. Sum of 178 alkyl homologs

Figure 19. Concentrations of phenanthrene and sum of 178 alkyl homologs in the tissue of *N. incisa* exposed to BRH suspended sediments for 42 days

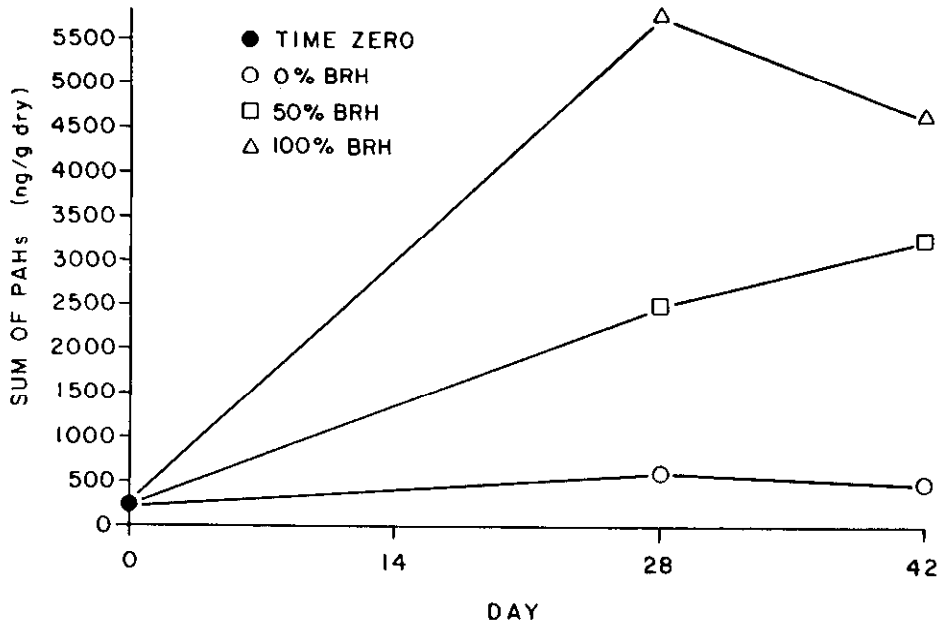


a. Fluoranthene

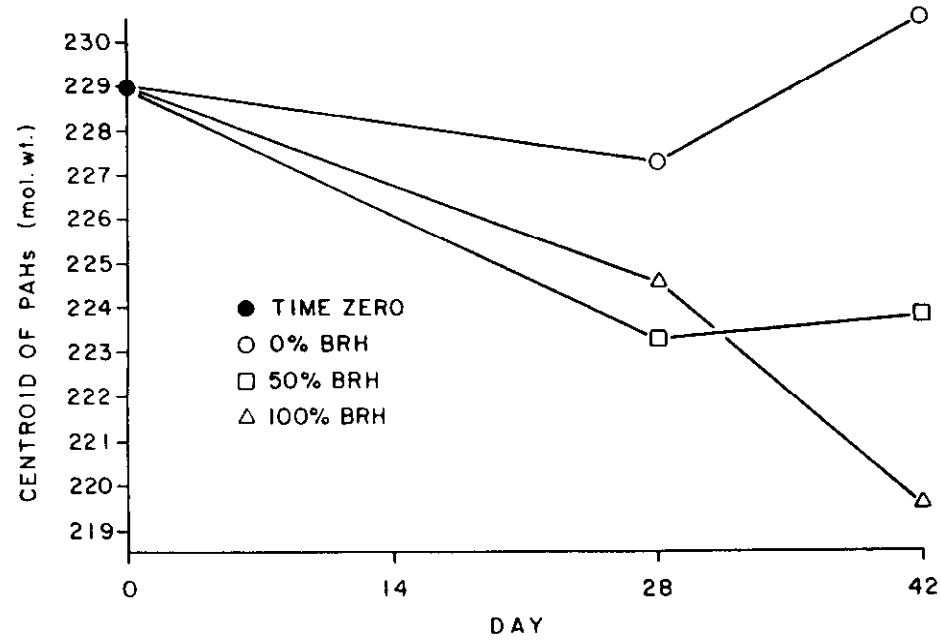


b. Benzo(a)pyrene

Figure 20. Concentrations of fluoranthene and benzo(a)pyrene in the tissue of *N. incisa* exposed to BRH suspended sediments for 42 days

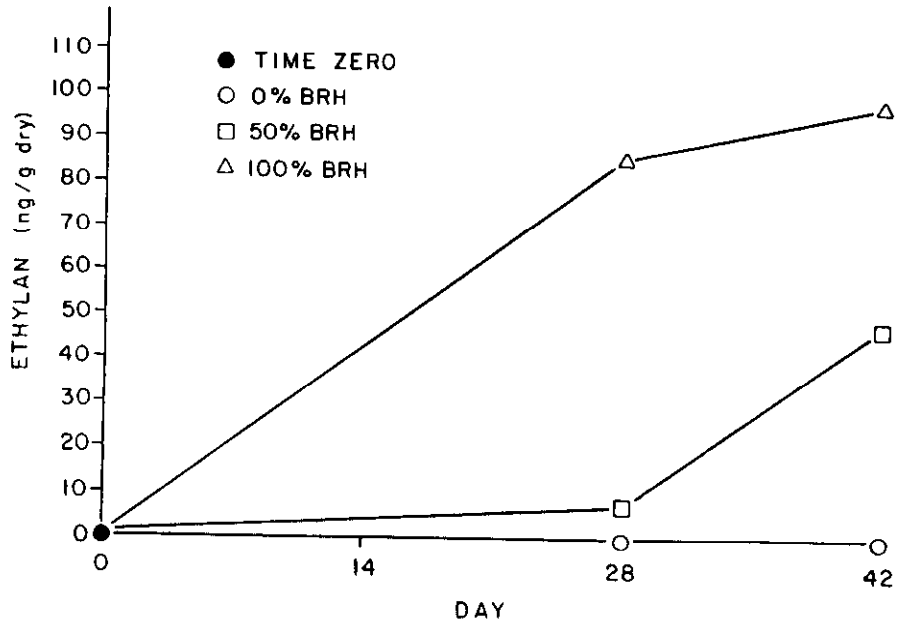


a. SUM of PAHs

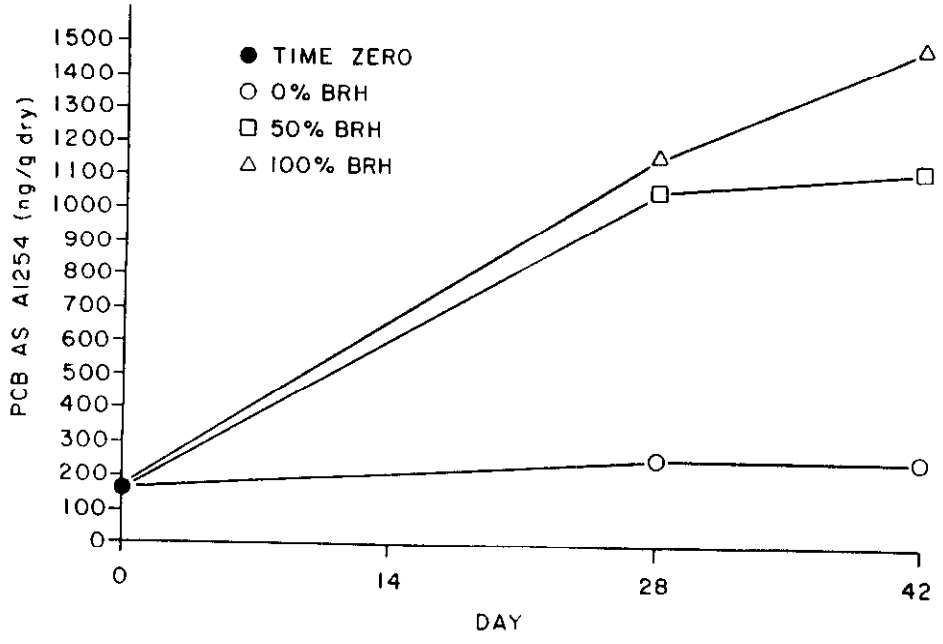


b. CENT of PAHs

Figure 21. Concentrations of SUM of PAHs and CENT of PAHs in the tissue of *N. incisa* exposed to BRH suspended sediments for 42 days

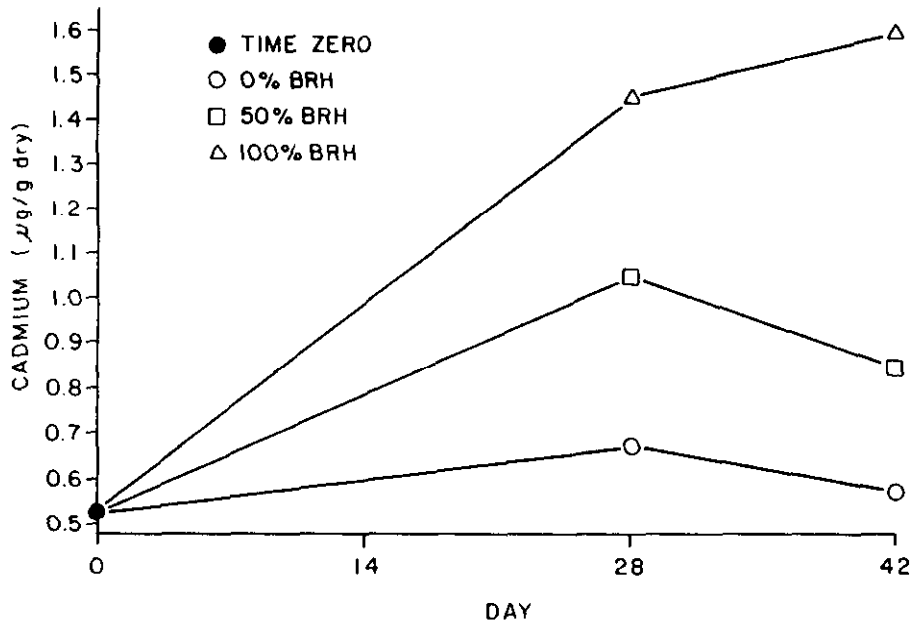


a. Ethylan

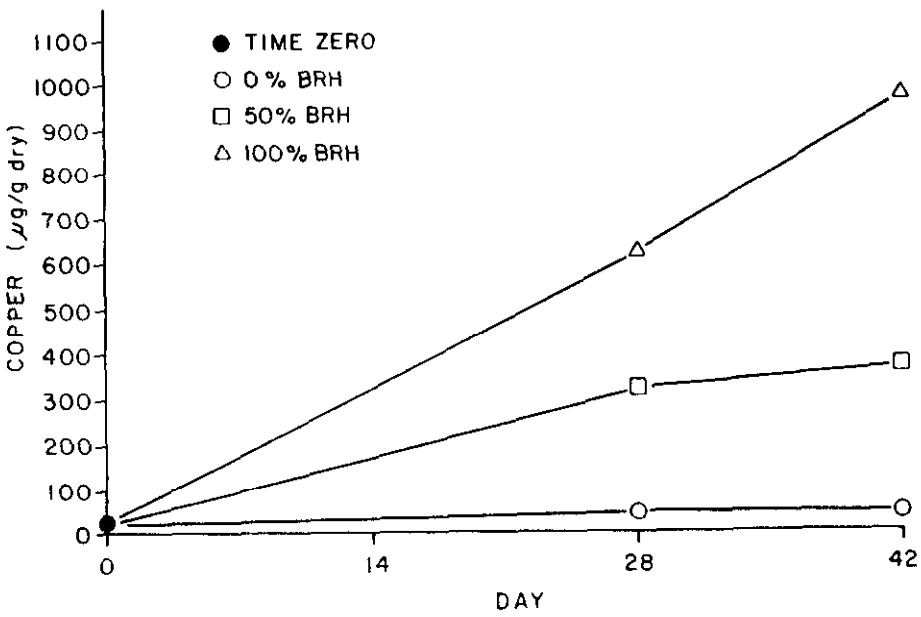


b. PCB as A1254

Figure 22. Concentrations of ethylan and PCB as A1254 in the tissue of *N. incisa* exposed to BRH suspended sediments for 42 days

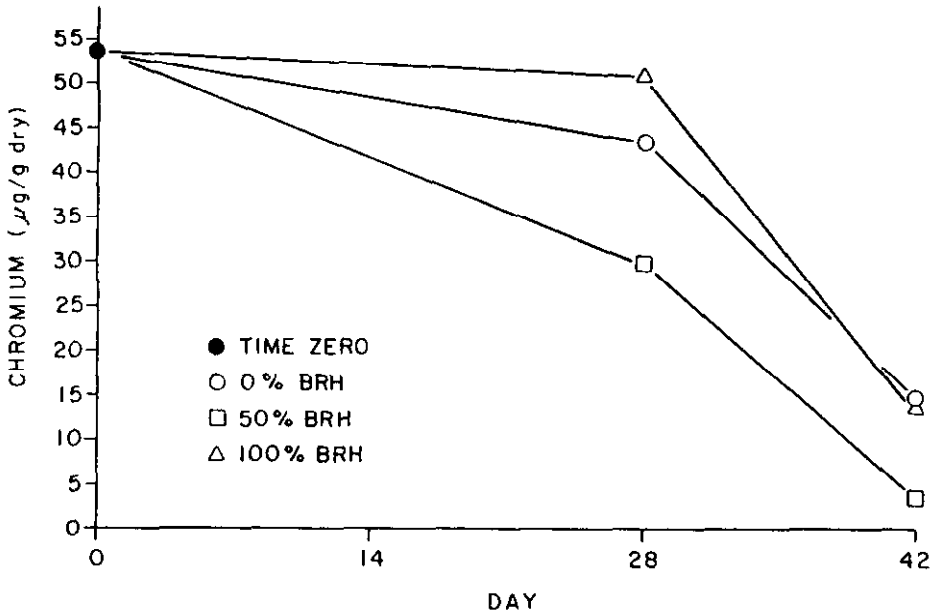


a. Cadmium

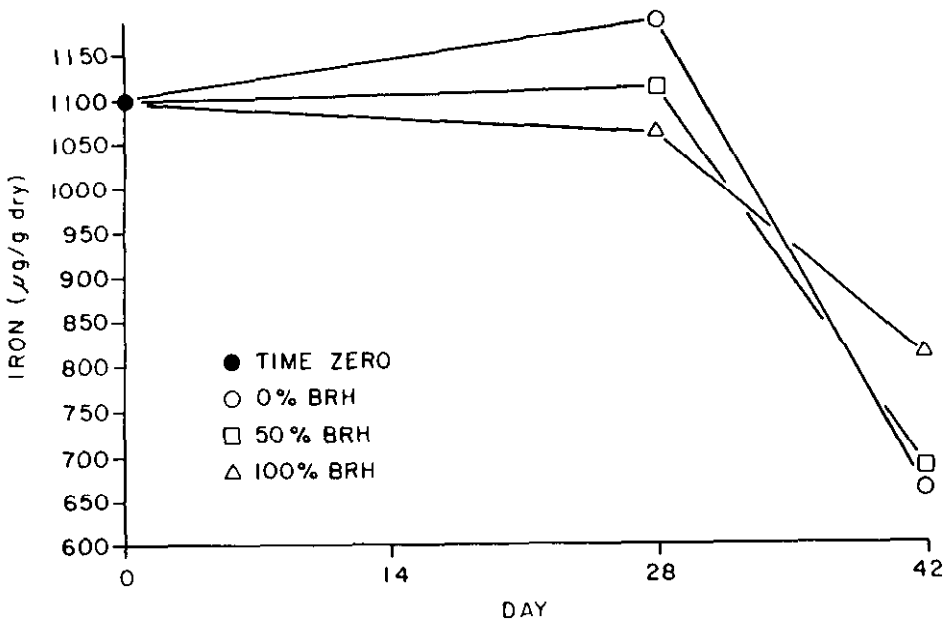


b. Copper

Figure 23. Concentrations of cadmium and copper in the tissue of *N. incisa* exposed to BRH suspended sediments for 42 days



a. Chromium



b. Iron

Figure 24. Concentrations of chromium and iron in the tissue of *N. incisa* exposed to BRH suspended sediments for 42 days

Table 12

Adenine Nucleotide Concentrations (μmol/g Wet Weight) in *M. edulis* from the Two Laboratory Experiments

Treatment % BRH	ATP	ADP	AMP	Total	AEC
		Experiment 1, Day 14			
0	5.01	1.23	0.07	6.31	0.89
50	3.83	1.19	0.13	5.15	0.86
100	3.68	0.83	0.05	4.56	0.90
		Experiment 2, Day 14			
0	4.26	1.64	0.38	6.28	0.81
10	3.29	1.76	0.60	5.65	0.73
30	3.09	1.80	0.65	5.55	0.71
		Experiment 2, Day 28			
0	3.93	1.41	0.22	5.56	0.83
10	4.07	1.40	0.25	5.71	0.83
30	3.90	1.61	0.35	5.86	0.80

These data were pooled and analyzed for correlations between BRH exposure concentrations and adenine nucleotide concentrations. The only significant relationship occurred between BRH exposure and the adenine nucleotide pool ($P = 0.001$, $r^2 = 0.94$). The other adenine variables and the AEC showed no significant relationship with BRH exposure concentrations.

97. *Nephtys incisa.* Adenine nucleotide concentrations were measured in whole worms exposed to BRH sediments in three laboratory experiments. The results of these measurements are presented in Table 13. These data are presented graphically in Figure 26. The data for day 28 from the second and third experiments were pooled and analyzed for correlations between BRH exposure concentrations and adenine nucleotide responses. There were no significant relationships between BRH exposure and adenine nucleotide pool concentrations, other adenine variables, or AEC.

Field

Exposure

98. *Mytilus edulis* exposures estimated from tissue residues. The first method used to estimate exposure conditions of *M. edulis* to BRH material in

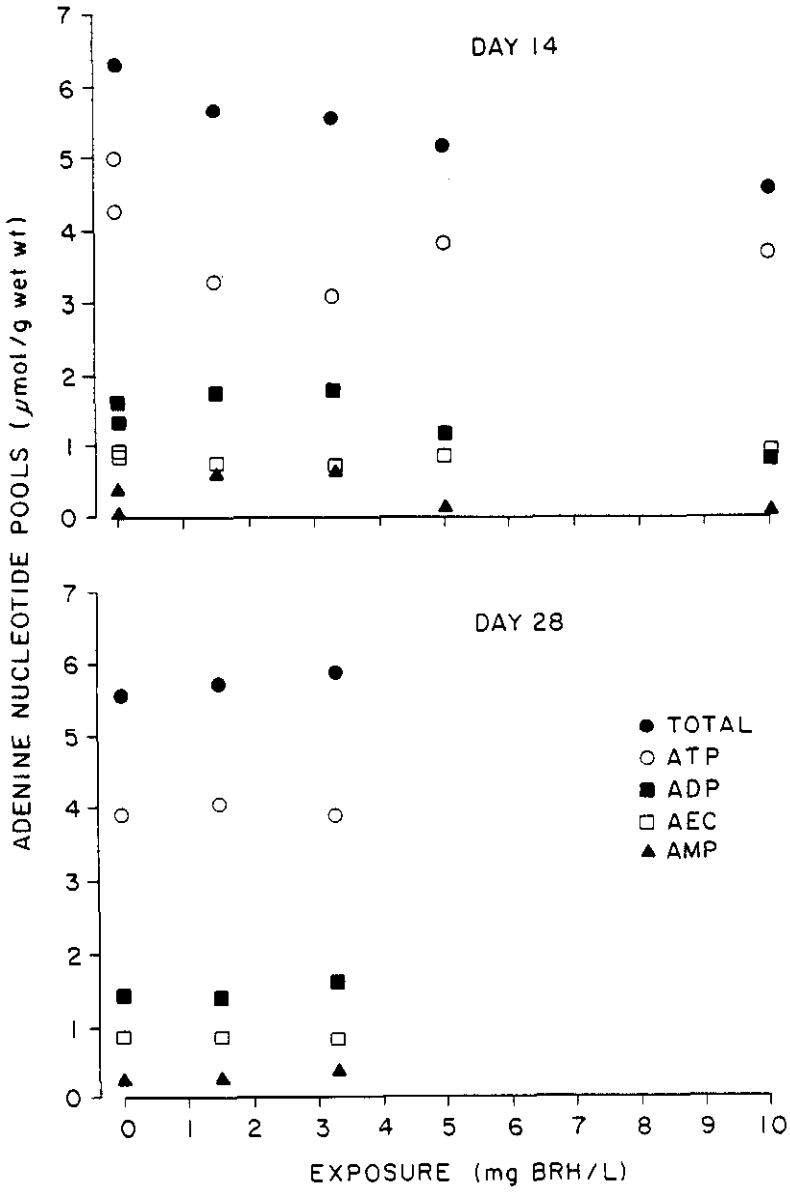


Figure 25. Response of adenine nucleotide pools in *M. edulis* to BRH exposure in laboratory experiments

CLIS involved the use of laboratory-generated relationships between PCB tissue residues and BRH exposures. There are several assumptions inherent in this process: mussels provided an integrated measure of exposure during each field deployment; mussels were at equilibrium with background BRH levels in the water column; and PCBs are a good chemical marker for BRH material. Based on the results of the laboratory experiments, each of these assumptions seems reasonable.

99. The predicted exposures for each station and collection date

Table 13

Adenine Nucleotide Concentrations (μmol/g Wet Weight) in *N. incisa* from the Three Laboratory Experiments

Treatment % BRH	ATP	ADP	AMP	Total	AEC
		Experiment 1, Day 10			
0	1.55	0.53	0.12	2.20	0.82
50	1.83	0.40	0.05	2.28	0.88
100	1.53	0.53	0.06	2.12	0.83
		Experiment 2, Day 28			
0	0.75	0.34	0.08	1.17	0.78
50	1.63	0.60	0.11	2.35	0.81
100	1.93	0.55	0.08	2.56	0.83
		Experiment 3, Day 28			
0	1.65	0.65	0.06	2.37	0.82
50	1.52	0.50	0.09	2.11	0.83
100	1.04	0.47	0.07	1.59	0.79
		Experiment 3, Day 42			
0	1.74	0.54	0.08	2.35	0.85
50	1.28	0.46	0.10	1.84	0.81
100	1.68	0.49	0.07	2.24	0.84

demonstrate several spatial and temporal trends (Table 14). Spatially, the data indicate a trend towards greater exposure near the CNTR station immediately following disposal. This is evidence by the elevated exposures at T = 0 (1000E > REFS) and T + 2 (400E > 1000E > REFS) towards the disposal mound. This pattern disappeared by T + 8, where exposures were nearly the same at the CNTR, 400E, and 1000E stations, with the REFS station being lower than the other three.

100. Temporally, the estimated BRH exposures decreased with increasing time from disposal. The maximum exposure occurred at the 400E station at T + 2. This value ranged between 1.4 and 0.8 mg/ℓ of BRH suspended sediment, depending on whether the background concentration at REFS was subtracted. By the next collection, T + 8, the maximum estimated exposure, also at 400E, decreased to between 0.7 and 0.3 mg/ℓ, half that of the previous collection.

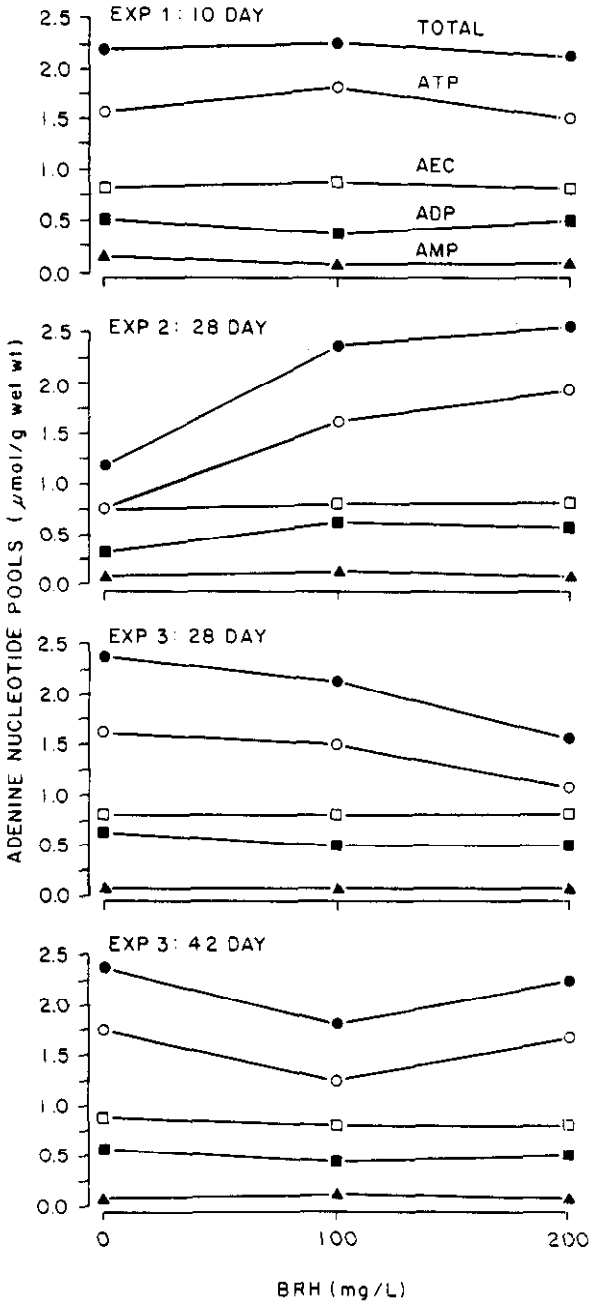


Figure 26. Response of adenine nucleotide pools in *N. incisa* to BRH exposure in laboratory experiments

Subsequent collections indicated a continued decrease to levels similar to those at the REFS station by T + 12.

101. Exposures estimated from water chemistry data. In addition to the estimates of BRH exposure based on mussel PCB tissue residues, a second

Table 14

Predicted BRH Suspended Material Sediment Exposure (mg/ℓ)
Required To Achieve the Measured Tissue Residue
Values of Mussels Deployed in CLIS*

Collection Cruise	Station	Estimated Exposure Range	
		High Value	Low Value
T - 04	CNTR	0.37	0.00
	400E	0.26	0.00
	1000E	0.38	0.00
	REFS	0.38	0.00
T = 0	1000E	1.04	0.56
	REFS	0.49	
T + 2	400E	1.39	0.79
	1000E	0.98	0.38
	REFS	0.60	
T + 8	CNTR	0.67	0.21
	400E	0.71	0.25
	1000E	0.60	0.14
	REFS	0.46	
T + 12	CNTR	0.61	0.06
	400E	0.64	0.09
	1000E	0.53	0.00
	REFS	0.55	
T + 15	CNTR	0.84	0.31
	400E	0.61	0.08
	1000E	0.53	
T + 21	CNTR	0.52	0.12
	400E	0.66	0.26
	1000E	0.55	0.15
	REFS	0.40	
T + 27	400E	0.52	0.09
	1000E	0.37	0.00
	REFS	0.43	

(Continued)

* Each estimate was calculated based on laboratory-generated PCB residue-exposure concentration relationships. The high value was determined from the actual mussel tissue residue concentration, while the low estimate was calculated after the REFS PCB residue was subtracted from the other stations during that collection period.

Table 14 (Concluded)

Collection Cruise	Station	Estimated Exposure Range	
		High Value	Low Value
T + 43	CNTR	0.33	0.06
	400E	0.31	0.04
	REFS	0.27	
T + 55	CNTR	0.52	0.00
	400E	0.42	0.00
	1000E	0.47	0.00
	REFS	0.53	
T + 116	CNTR	0.30	0.00
	400E	0.34	0.00
	1000E	0.43	0.01
	REFS	0.42	

estimate was made using PCB and copper concentrations in whole water samples taken postdisposal. The data indicate spatial and temporal trends similar to those obtained from the tissue residue estimates (Table 15).

102. Spatially, the sample collected on 7 June 1983 showed the highest BRH estimate (based on copper) at the CNTR station, followed by lower concentrations at 400E and 1000E stations, and the lowest levels at REFS. The estimate based on PCB concentrations indicated the CNTR station was elevated compared with the REFS station. The same pattern was observed in both the copper and PCB estimates for the 21 July 1983 sample. A decreasing concentration of BRH material was estimated moving away from the CNTR station.

103. On a temporal scale, the BRH concentration (copper data) decreased by about half from June to July (high estimate). After this collection, however, the copper-based BRH estimates fluctuated over time with the December 1983 and June 1984 values higher than the September 1983 concentrations. This pattern over time may be more reflective of CLIS than of actual BRH levels because these estimates (high value) were based on the absolute copper levels at each location. Inspection of the low estimate indicated a more distinct pattern over the same time period. The BRH levels were highest immediately after the disposal operation (June 1983) and generally decreased with increasing time. The low estimate provided here is more a measure of relative difference between the stations, after background Long Island Sound concentrations are subtracted (REFS). When trying to discern temporal trends, this estimate may be more appropriate.

104. The pattern of BRH exposure based on PCB water concentrations was very similar to that of copper. The highest value was detected at the CNTR station in June 1983 and decreased both spatially and temporally with increasing time. In addition, the high estimates did not show the same variability over time that the copper data did. This may indicate that PCB concentrations in Long Island Sound were most constant over time and thus BRH estimates based on PCB concentrations were less influenced by background fluctuations.

105. *Nephtys incisa* exposure estimated from tissue residues. The first method used to estimate exposure conditions of *N. incisa* to BRH material in CLIS involved the laboratory-generated relationships between PCB tissue residues and BRH exposures. Using this relationship and the PCB tissue residues in field-collected *N. incisa*, estimates of field BRH exposure concentrations

Table 15

Predicted BRH Suspended Sediment Exposure (mg/ℓ) Based on PCB and Copper Whole Water Chemistry Data*

Collection Cruise	Station	Estimate Using Copper		Estimate Using PCB	
		High Value	Low Value	High Value	Low Value
T + 2	CNTR	1.30	0.71	1.05	0.69
	400E	1.12	0.53	--	--
	1000E	1.14	0.55	--	--
	REFS	0.59	0.00	0.36	0.00
T + 9	CNTR	0.62	0.26	0.19	0.11
	400E	0.49	0.13	--	--
	1000E	0.41	0.05	--	--
	REFS	0.36	0.00	0.08	0.00
T + 15	CNTR	--	--	0.17	0.07
	400E	--	--	0.21	0.11
	1000E	--	--	0.16	0.06
	REFS	--	--	0.10	0.00
T + 15	CNTR	--	--	--	--
	400E	0.72	0.22	--	--
	1000E	--	--	--	--
	REFS	0.50	0.00	--	--
T + 28	CNTR	--	--	0.05	0.00
	400E	1.13	0.37	0.08	0.00
	1000E	--	--	0.09	0.00
	REFS	0.91	0.00	0.09	0.00
T + 55	CNTR	--	--	--	--
	400E	1.00	0.09	--	--
	1000E	--	--	--	--
	REFS	0.91	0.00	--	--

* Each estimate was calculated through division of the concentration of PCB or copper present in the field by the concentration of that material present in the BRH barrel material (6,910 ng/g and 2,900 µg/g for PCB and copper, respectively). The high value was determined from the actual whole water concentration while the low value was calculated after the REFS values were subtracted.

were calculated. There are several assumptions in this approach: *N. incisa* provides an integrated measure of exposure; *N. incisa* tissue residues were at steady-state with BRH exposure concentrations at the time of sampling; and PCBs are a good chemical marker for BRH sediments. Based on the results of the laboratory experiment, each of these assumptions seems reasonable.

106. The estimated exposures resulting from this approach are presented as milligrams per litre BRH for each station and collection date in Table 16.

Table 16

Estimated Concentrations of BRH Sediment (mg/ℓ) Suspended at Sediment-Water Interface Based on PCB Concentrations in Field-Collected *N. incisa*

Date	Station			
	CNTR	400E	1000E	REFS
17 Apr 82	--	0	--	0
16 Nov 82	--	0	--	2
16 Feb 83	--	9	--	3
12 Apr 83	--	15	--	8
02 Jun 83	--	95	43	2
03 Jul 83	--	114	44	2
06 Sep 83	--	131	88	12
29 Nov 83	--	51	26	0
20 Mar 84	47	38	10	0
16 Oct 84	53	29	10	3
24 Jan 86	76	5	4	0

Nephtys incisa at CNTR were buried during disposal of the dredged material and did not recolonize the dredged material mound until the spring of 1984. When the worms recolonized the mound, sampling began. The data in Table 16 display clear spatial and temporal trends. The highest estimates were in the immediate vicinity of the disposed BRH material (400E) during the summer of 1983. There was a decrease in exposure at 400E and 1000E in 1984 and 1985.

107. *Nephtys incisa* exposure estimates from physical data. Benthic exposure at the FVP disposal site can occur through both the suspended and bedded sediments. This section describes predictions of the maximum upper

bound suspended sediment concentrations from 1 m above the bottom to the sediment-water interface. This calculation is based upon the assumption that the sediment-water interface consists totally of BRH sediment and that the suspended solids at the sediment-water interface consist totally of BRH sediment and that the contaminant concentrations are similar to those found in the dredged material prior to disposal.

108. TSS concentrations were measured at the FVP site at 1 m above the sediment-water interface with an in situ monitoring platform (Bohlen and Winnick 1986). Although there is considerable variation in the data through one tidal cycle, average background suspended solids were estimated to be 10 mg/ℓ, while during typical storm conditions suspended solids concentrations averaged 30 mg/ℓ for periods of 4 to 7 days (Munns et al. 1986).

109. Using an acoustic profilometer, the suspended sediment concentrations at 1 m above the bottom were found to increase exponentially to the sediment-water interface. The upper and lower limits for this increase are 10× and 1×, respectively, depending on hydrographic conditions (Bohlen and Winnick 1986). These data, in conjunction with suspended sediment data for 1 m above the bottom, can be used to predict the suspended solids concentrations at the sediment-water interface.

110. For example, the suspended solids concentration under background conditions (10 mg/ℓ) would increase to 100 mg/ℓ for the 10× enrichment at the sediment-water interface and decrease to 10 mg/ℓ for the quiescent conditions. Likewise, under storm conditions (30 mg/ℓ), the sediment-water interface suspended solids concentrations would range from 300 to 30 mg/ℓ for the 10× and 1× enrichments, respectively (Figure 27). These conditions represent the maximum upper bound exposures that would be expected to occur at the sediment-water interface and could be made using predisposal, site characterization data.

111. A more probable estimate is provided by using contaminant concentrations present in the sediment after disposal. It is this material that will be resuspended and transported as suspended solids to populations outside the disposal site. Assuming that resuspended surficial sediments are the source of contaminants for the suspended sediments, the maximum upper bound estimates can be adjusted to reflect the spatial and temporal changes in contaminant concentration. These changes are represented as percentages of BRH sediment in the 0- to 2-cm surface layer at CNTR, 200E, 400E, and 1000E from

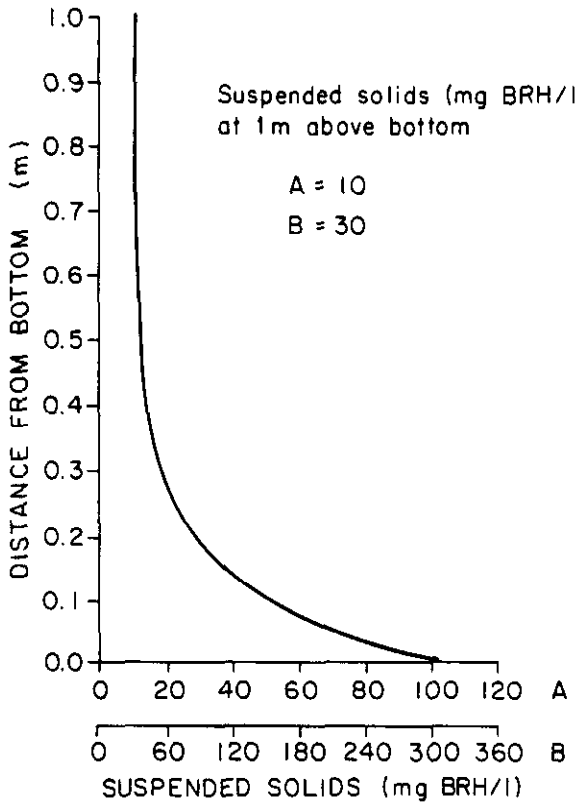


Figure 27. Suspended sediment concentrations from 1 m above the bottom to the sediment-water interface for storm and background conditions

June 1983, immediately after disposal, to October 1985 (Table 17). The combination of these percentages and the TSS concentrations predicted for the sediment-water interface results in concentrations of BRH suspended sediments at the sediment-water interface for each station and sampling date (Table 18).

112. The sediment samples used for the percent calculations were not replicated; therefore, no variability estimates are available. However, certain trends in the data are evident (Table 17). The percentages of BRH sediment (<50 percent) at CNTR and 200E were low compared with the barrel sediments collected predisposal. There is a gradient of BRH material that is a function of both distance from the center of the mound and of time from disposal. BRH sediment concentrations were highest at CNTR and 200E immediately after disposal and decreased significantly through October 1984. Concentrations were elevated in December 1984 at CNTR and 200E and again in October 1985 at 200E. The BRH concentrations at 400E also decreased through time and, after December 1983, were the same or higher than those at 1000E.

113. The 1- to 2-percent BRH sediment calculated for 1000E represents a quantitatively measured elevation above background and is supported by tissue residue data for *N. incisa*. This contamination could have resulted from the

Table 17

Percent BRH Sediment in the Surficial Sediments at the FVP Disposal Site

Date	Station			
	CNTR	200E	400E	1000E
Jun 83	44.5	41.1	12.5	1.8
Jul 83	15.0	37.4	3.3	1.6
Sep 83	32.0	36.7	4.9	2.0
Dec 83	32.8	36.1	9.5	4.4
Mar 84	4.4	2.2	1.9	1.8
Jun 84	9.5	15.6	0.5	0.7
Sep 84	10.0	0.8	3.5	0.5
Oct 84	2.6	--	0.2	1.6
Dec 84	35.1	11.3	0.0	1.0
Oct 85	0.2	21.0	0.0	0.0

Table 18

Concentration of BRH (mg/ℓ) at the Sediment-Water Interface for TSS Concentrations of 30 mg/ℓ and 10 mg/ℓ and an Enrichment of 10×*

Date	Station							
	CNTR		200E		400E		1000E	
	300	100	300	100	300	100	300	100
Jun 83	133.5	44.5	123.3	41.1	37.5	12.5	5.4	1.8
Jul 83	45.0	15.0	112.2	37.4	9.9	3.3	4.8	1.6
Sep 83	96.0	32.0	110.1	36.7	14.7	4.9	6.0	2.0
Dec 83	98.4	32.8	108.3	36.1	28.5	9.5	13.2	4.4
Mar 84	14.2	4.4	6.6	2.2	4.7	1.9	5.4	1.8
Jun 84	28.5	9.5	46.8	15.6	1.5	0.5	2.1	0.7
Sep 84	30.0	10.0	2.4	0.8	10.5	3.5	1.5	0.5
Oct 84	7.8	2.6	--	--	0.6	0.2	4.8	1.6
Dec 84	105.3	35.1	33.9	11.3	0.0	0.0	3.0	1.0
Oct 85	0.6	0.2	63.0	21.0	0.0	0.0	0.0	0.0

* BRH concentrations for the 1× enrichment can be obtained by dividing the tabular values by 10.

dispersion of dredged material during disposal, the errant disposal of BRH material in the vicinity of 1000E, or the continuous transport of contaminated material from the disposal mound.

114. The estimates of exposure to BRH material at the sediment-water interface derived from tissue concentrations of PCB and from the maximum upper bound predictions agreed well. The exposure estimates based on the chemistry of the 0- to 2-cm surface sediments were low. If the exposure estimates based on tissue concentrations of PCB are accepted as a valid check on the exposure estimates from the physical data, it is concluded that the higher estimates of exposure are accurate. The simplest explanation is that the 0- to 2-cm sampling procedure integrates clean and contaminated sediments, thus underestimating the actual exposures experienced by the worms. The data suggest that the worms were exposed to a thin, surface layer of contaminated sediment.

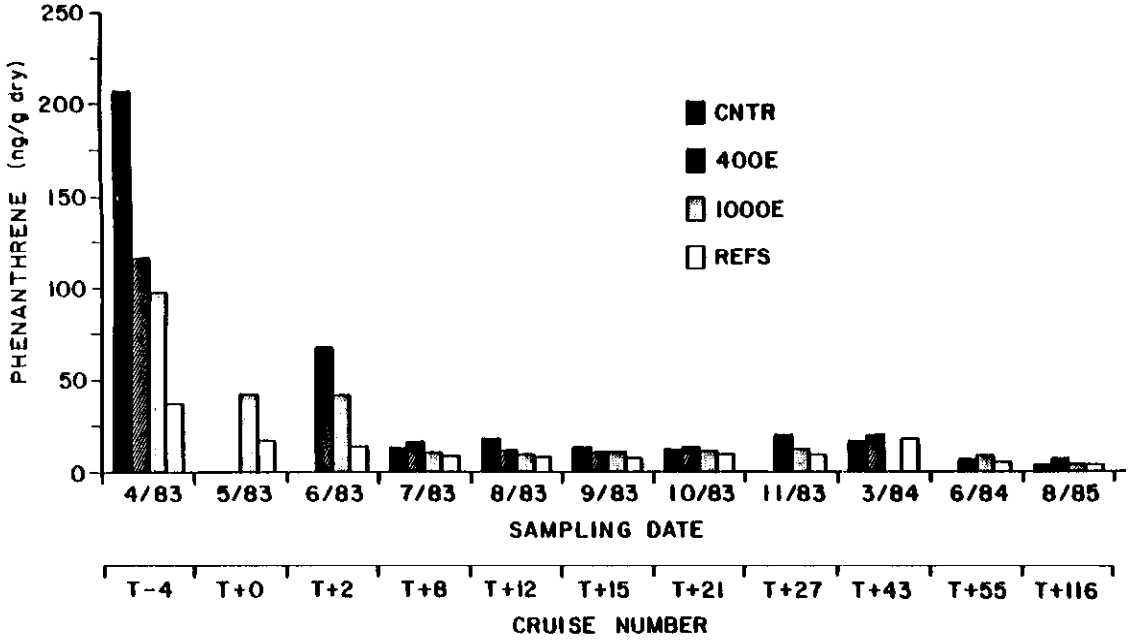
Tissue residues

115. *Mytilus edulis.* The tissue residue levels for the mussels collected during the course of the FVP study are presented graphically in Figures 28-33 for each of the 12 selected organic, inorganic, and summary statistic chemical contaminants. The raw data shown on these figures are included in Appendix B.

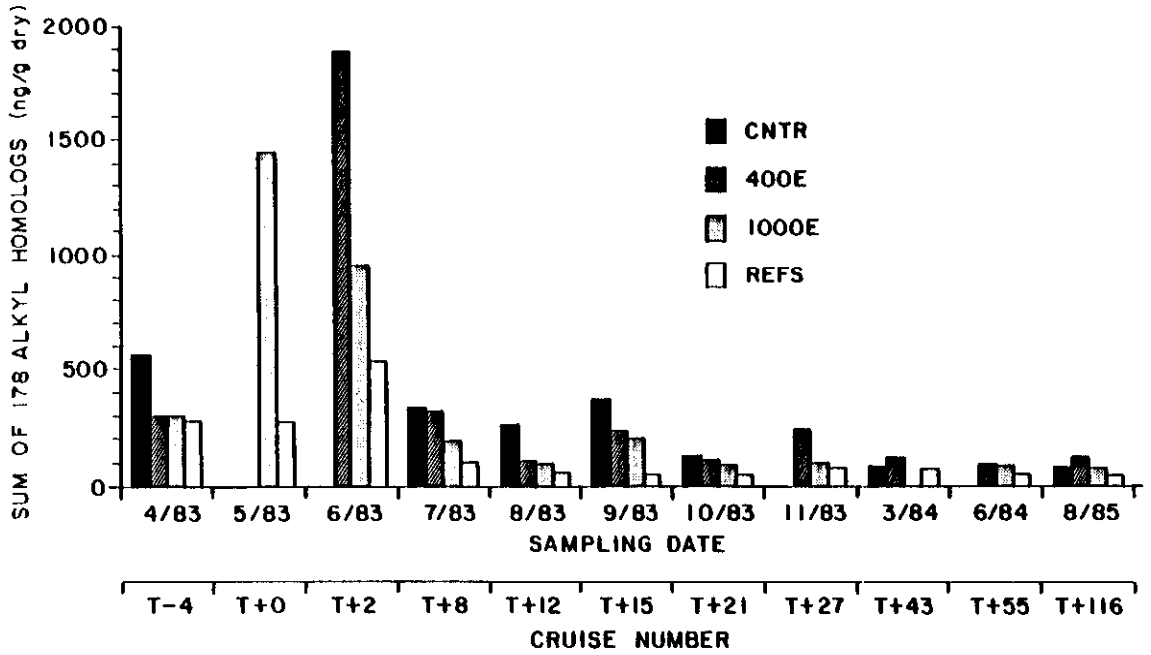
116. The PCB, ethylan, and PAH residues increased during the disposal operation. After completion of the disposal, tissue residues decreased to concentrations similar to those from predisposal deployments. The summary statistic, SUM, reflected the same pattern as most of the PAH compounds.

117. A consistent pattern emerged when the spatial component of the organic residue data was considered within a sampling date. *Mytilus edulis* were deployed during the actual disposal operation, and were collected at T + 0 and T + 2. For the T + 0 collection, only the 1000E and REFS stations were recovered. The tissue residue concentration for each organic compound was uniformly higher at the 1000E station than REFS. The T + 2 collection included data from three stations, 400E, 1000E, and REFS. Once again, a consistent pattern is seen in the residue data with mussels at 400E exhibiting the highest concentrations for each compound, followed by the 1000E and REFS stations. After the completion of disposal, the differences in tissue residue concentrations between stations decreased dramatically.

118. The tissue residue data for metals did not provide as clear a picture of the disposal operation as the organic residues. In general, metal

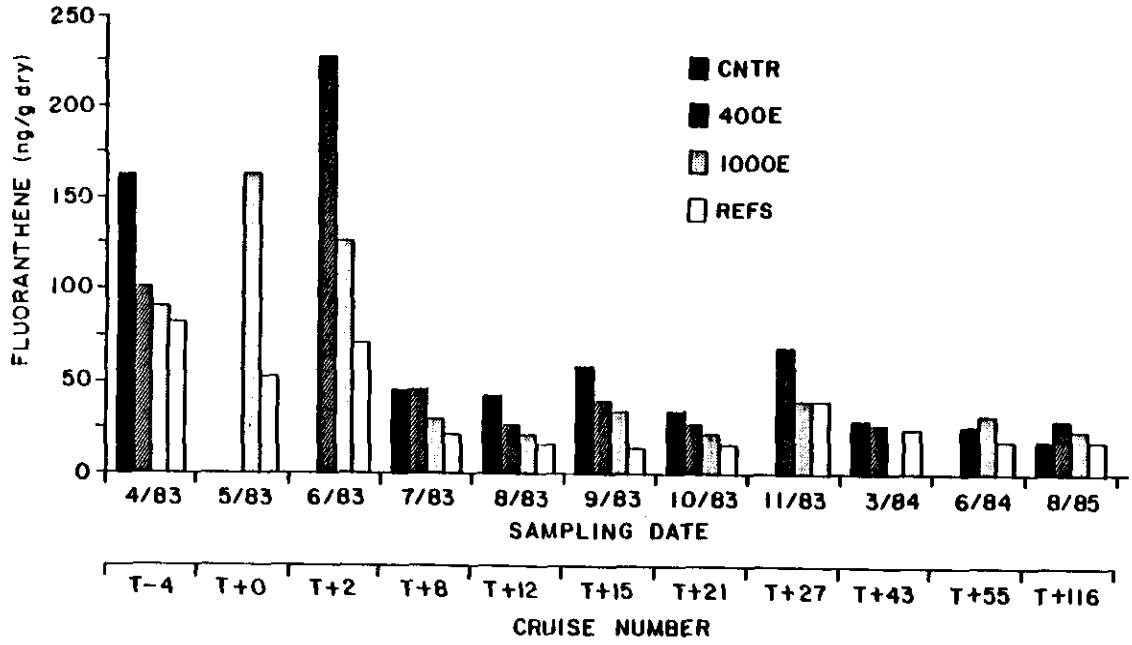


a. Phenanthrene

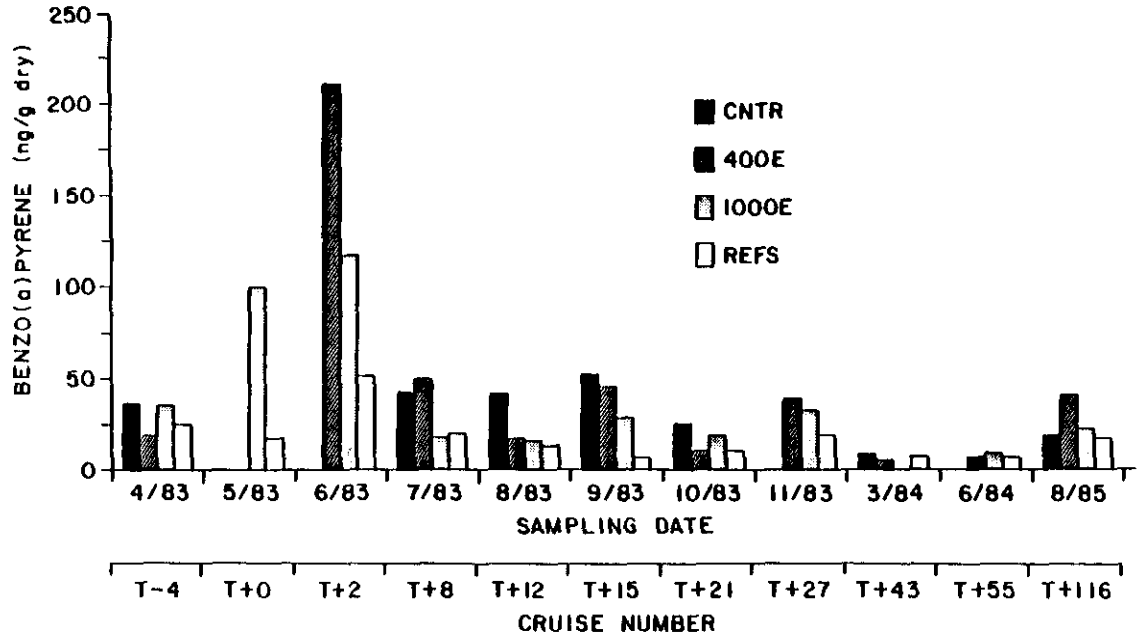


b. Sum of 178 alkyl homologs

Figure 28. Concentrations of phenanthrene and the sum of 178 alkyl homologs in the tissues of *M. edulis* exposed at the specified FVP stations and sampling dates

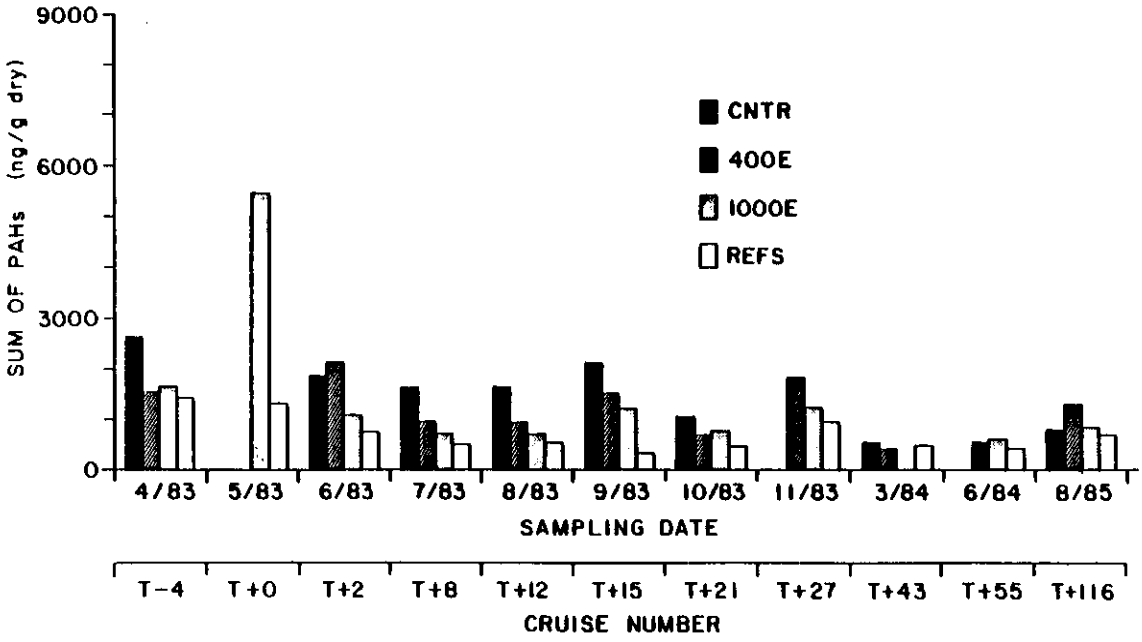


a. Fluoranthene

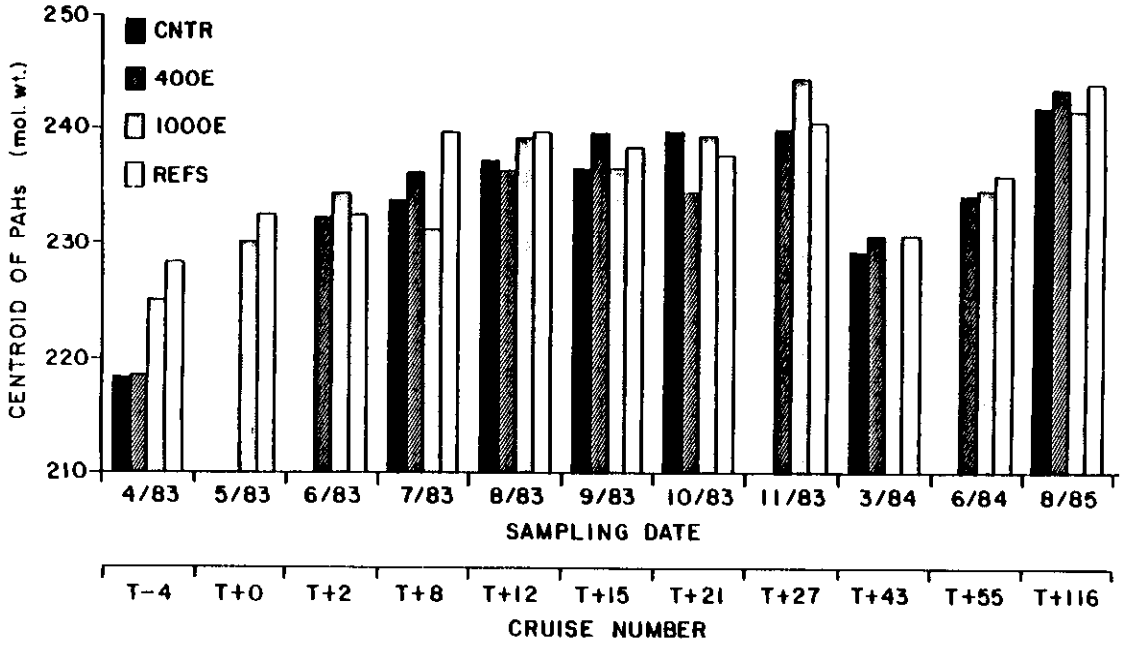


b. Benzo(a)pyrene

Figure 29. Concentrations of fluoranthene and benzo(a)pyrene in the tissues of *M. edulis* exposed at the specified FVP stations and sampling dates

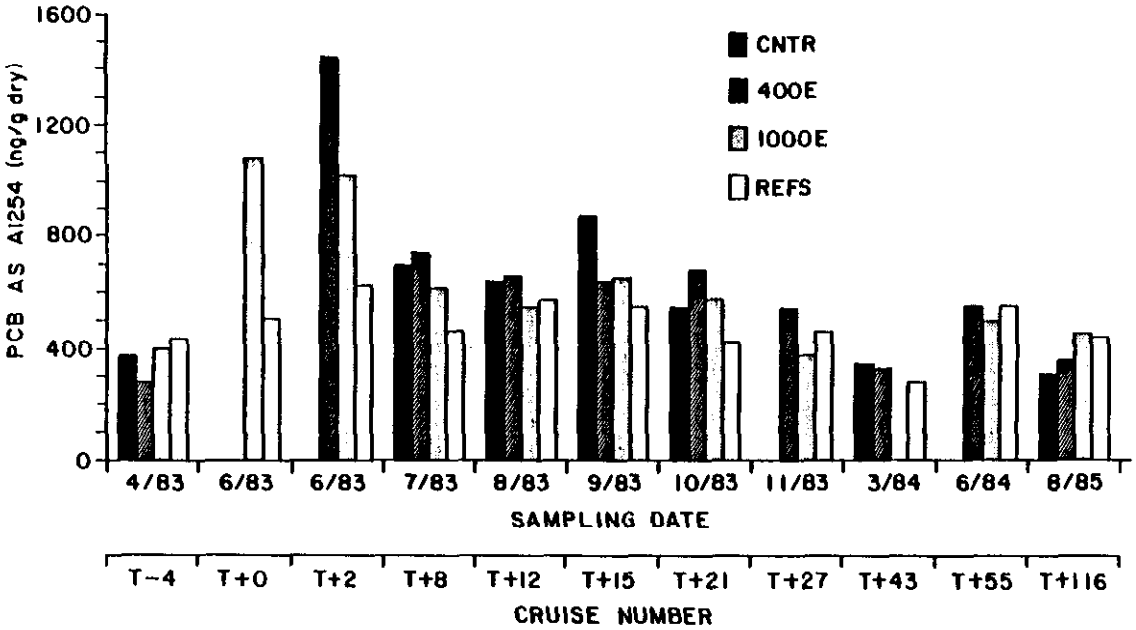


a. SUM of PAHs

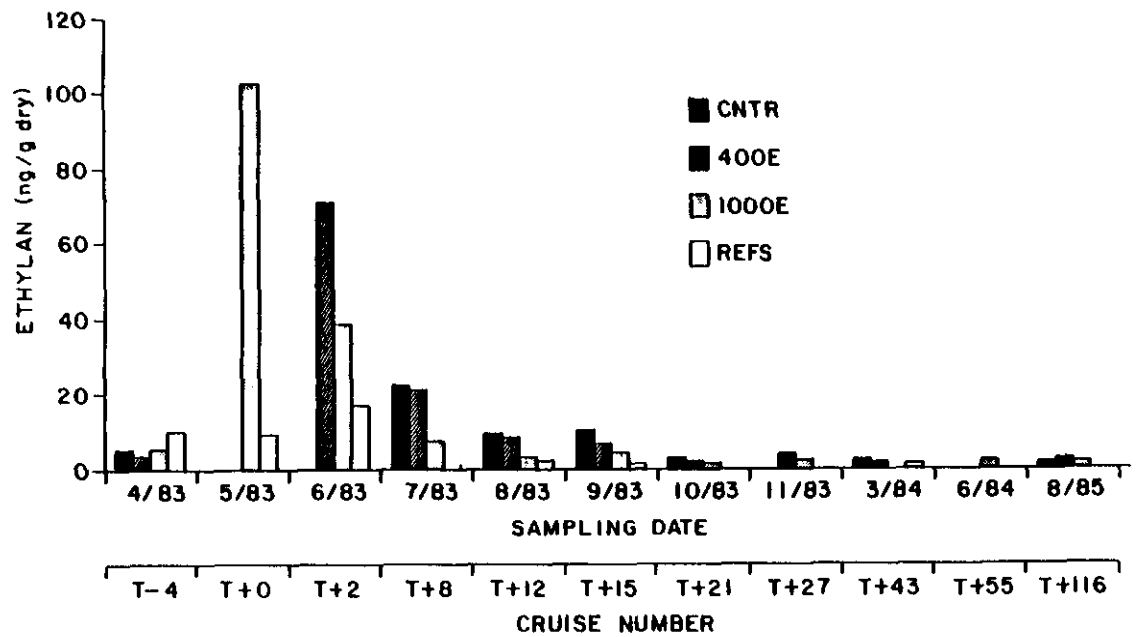


b. CENT of PAHs

Figure 30. Concentrations of the SUM of PAHs and CENT of PAHs in the tissues of *M. edulis* exposed at the specified FVP stations and sampling dates

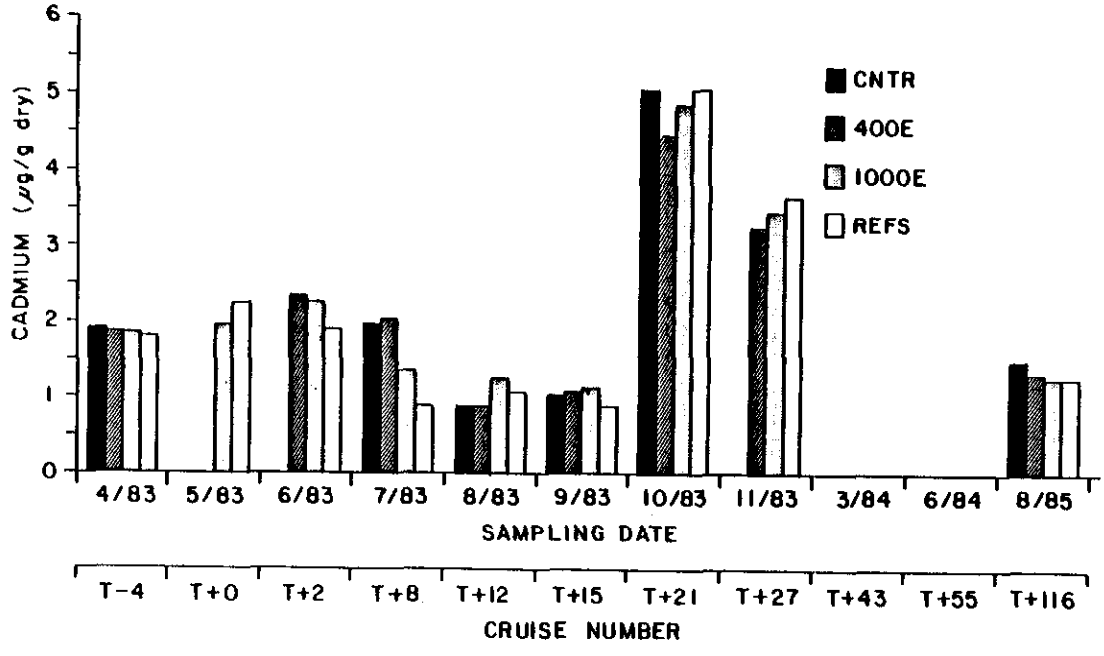


a. PCB as A1254

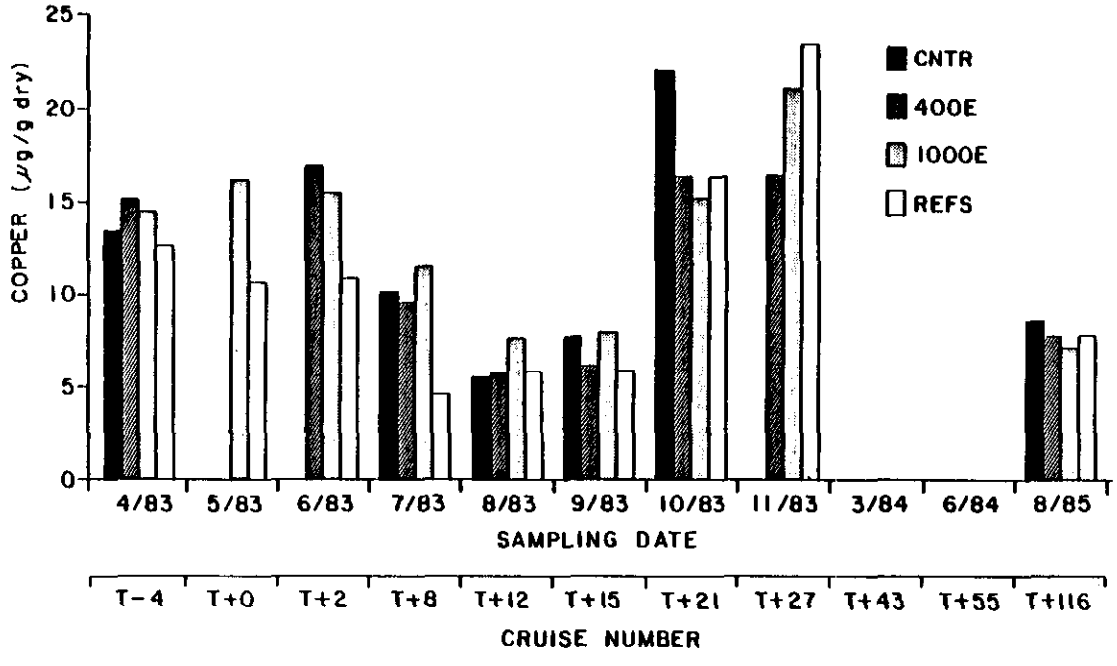


b. Ethylan

Figure 31. Concentrations of PCB as A1254 and ethylan in the tissues of *M. edulis* exposed at the specified FVP stations and sampling dates

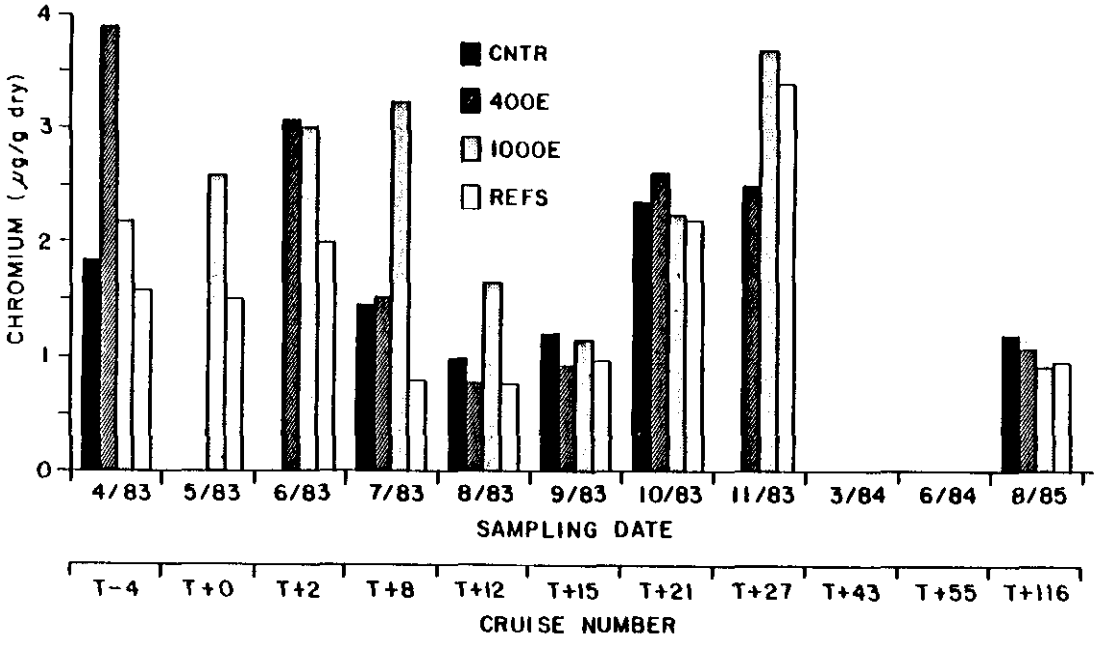


a. Cadmium

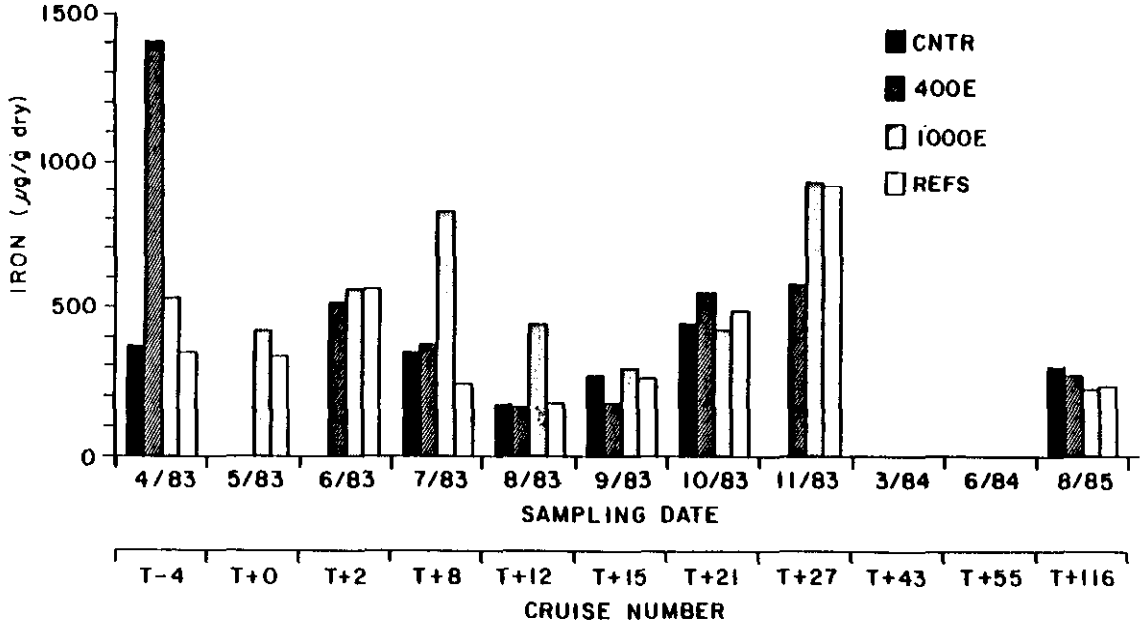


b. Copper

Figure 32. Concentrations of cadmium and copper in the tissues of *M. edulis* exposed at the specified FVP stations and sampling dates



a. Chromium



b. Iron

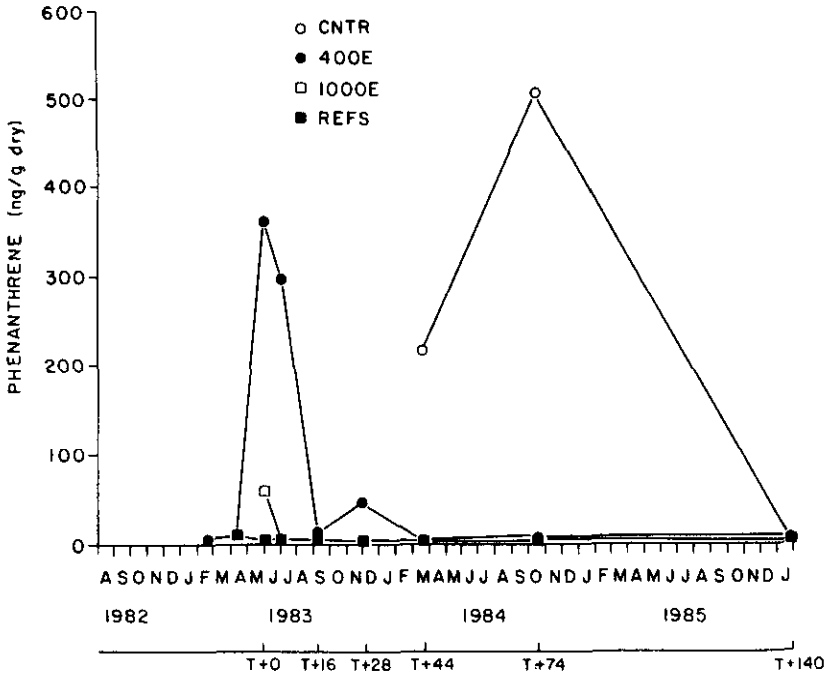
Figure 33. Concentrations of chromium and iron in the tissues of *M. edulis* exposed at the specified FVP stations and sampling dates

residue concentrations increased slightly during the disposal operation, after which they decreased to levels well below those present during the predisposal collection (T - 4). Metal concentrations were elevated in *M. edulis* collected in October and November 1983 (T + 21, T + 27), well above those present even during the disposal operation (T + 0, T + 2). The October and November samples consisted of organisms that had been deployed at the FVP site for 7 and 3 months, respectively. One possible explanation for the difference between elevated metal and organic tissue residue patterns may be that mussels require a long period of time to reach steady-state with respect to metal concentrations. Comparing the organic and metal residue data from the field suggests that organic tissue residues present a better picture of the disposal operation at the FVP disposal site.

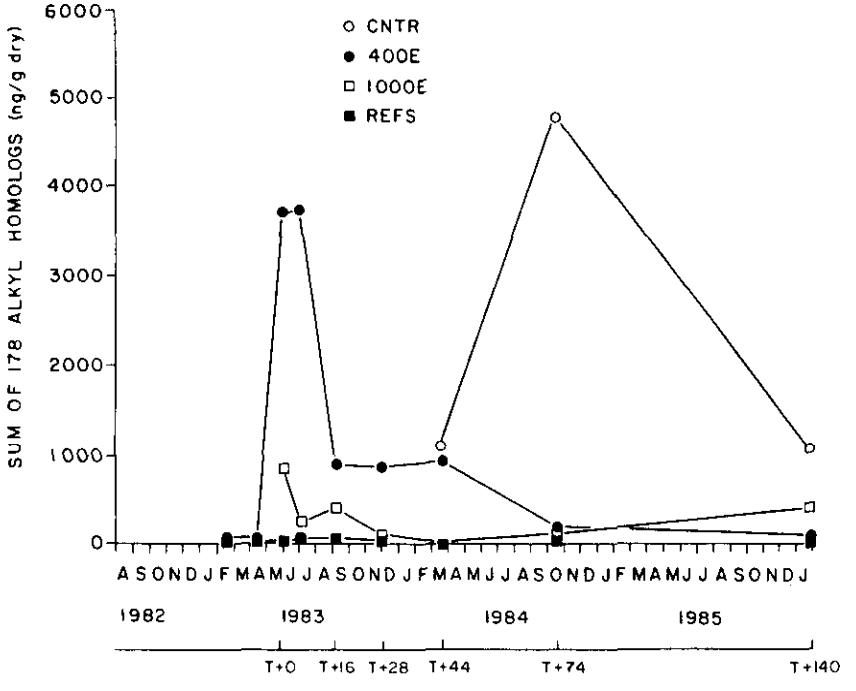
119. *Nephtys incisa.* The tissue concentrations for the *N. incisa* collected at the CLIS site during the FVP study are presented graphically for each of the 12 selected chemical variables in Figures 34-39. The raw data shown on these figures are included in Appendix B.

120. Clear spatial and temporal patterns of tissue concentrations of PCBs and PAHs were found. Highest tissue concentrations were determined at station 400E with lowest concentrations at station REFs. When *N. incisa* recolonized the dredged material site at station CNTR in the spring of 1984, the tissue concentrations of PCBs in these worms were comparable with those found at 400E immediately postdisposal.

121. The temporal patterns of the field tissue residue show a rapid increase in organic residue values during and immediately postdisposal at 400E and at 1000E. The PAH residues for *N. incisa* showed an increase immediately postdisposal. This was followed by a rapid decline during July and August. The phenanthrene residue value returned to background levels by September, but the higher molecular weight PAH tissue residues tended to remain at approximately 25 percent of their maximum values for an additional year. The PCB residues at 400E increased rapidly immediately after disposal and, unlike the PAHs, remained elevated through September and declined only 50 percent by December 1983. Unlike the PAHs, PCB residues increased 2.5 times REFS at 1000E postdisposal and remained elevation above REFS until October 1984. There were no clear temporal or spatial patterns for inorganic tissue residues for *N. incisa* from the field.

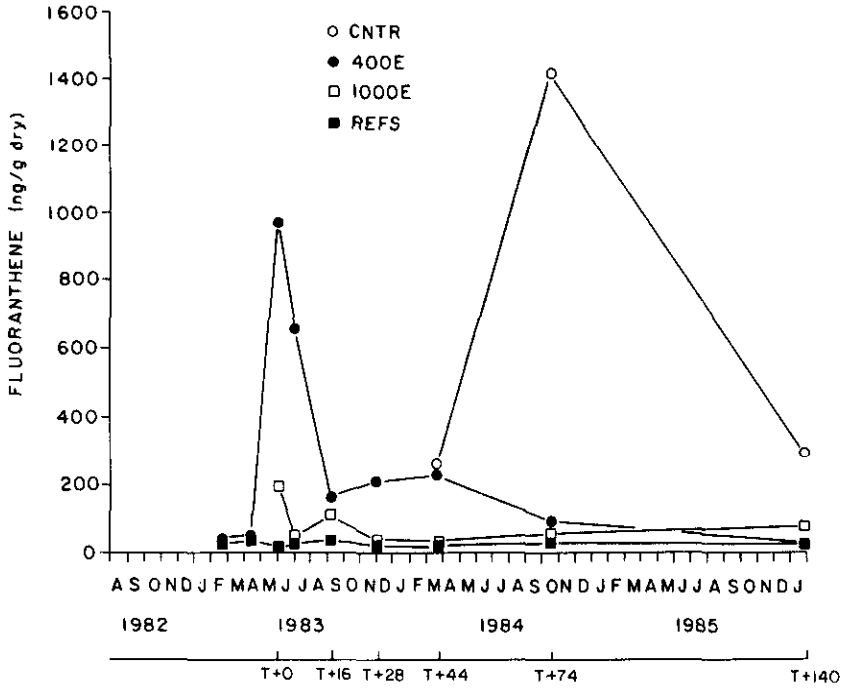


a. Phenanthrene

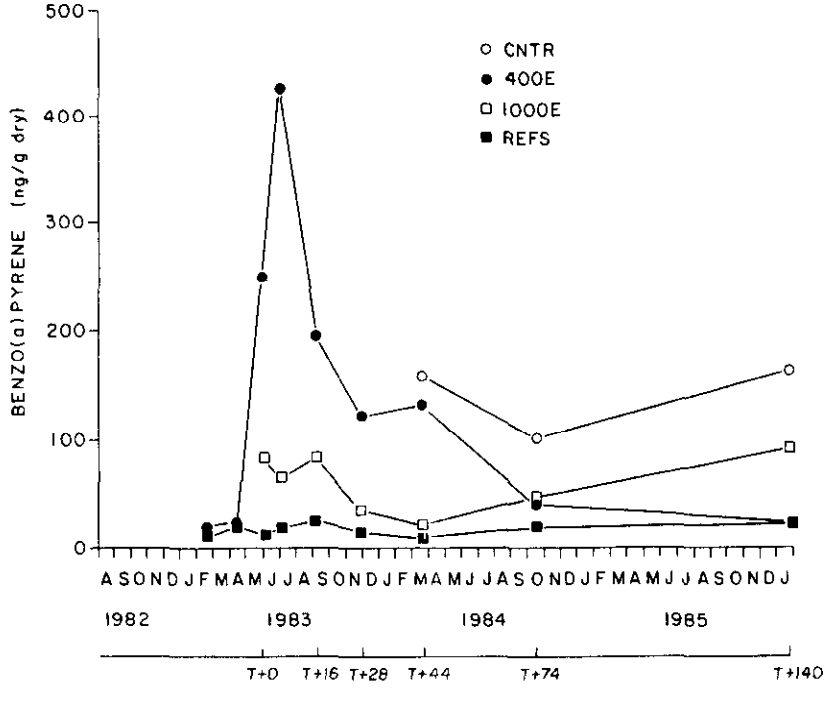


b. Sum of 178 alkyl homologs

Figure 34. Concentrations of phenanthrene and the sum of 178 alkyl homologs in the tissues of *N. incisa* collected at the specified FVP stations and sampling dates

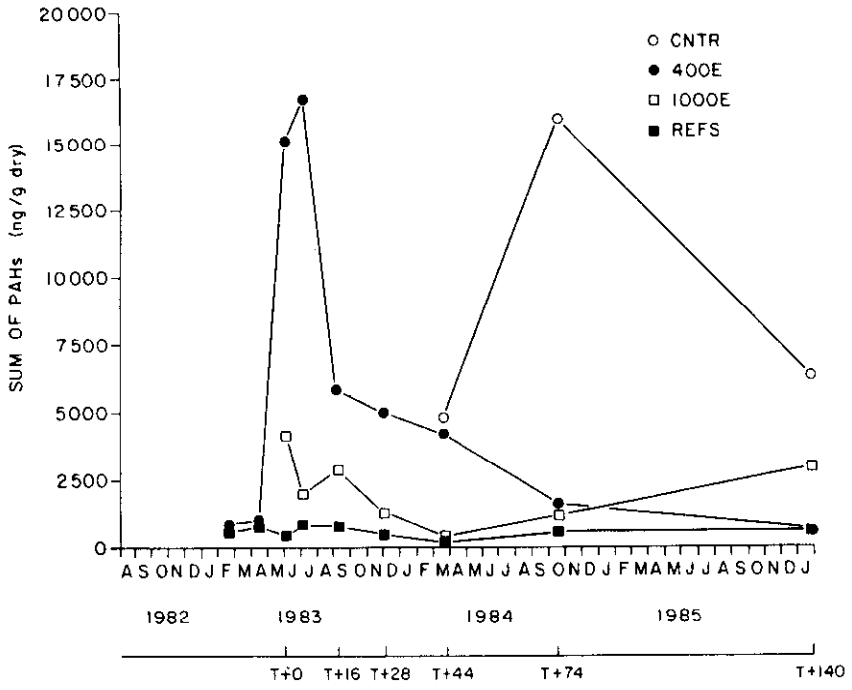


a. Fluoranthene

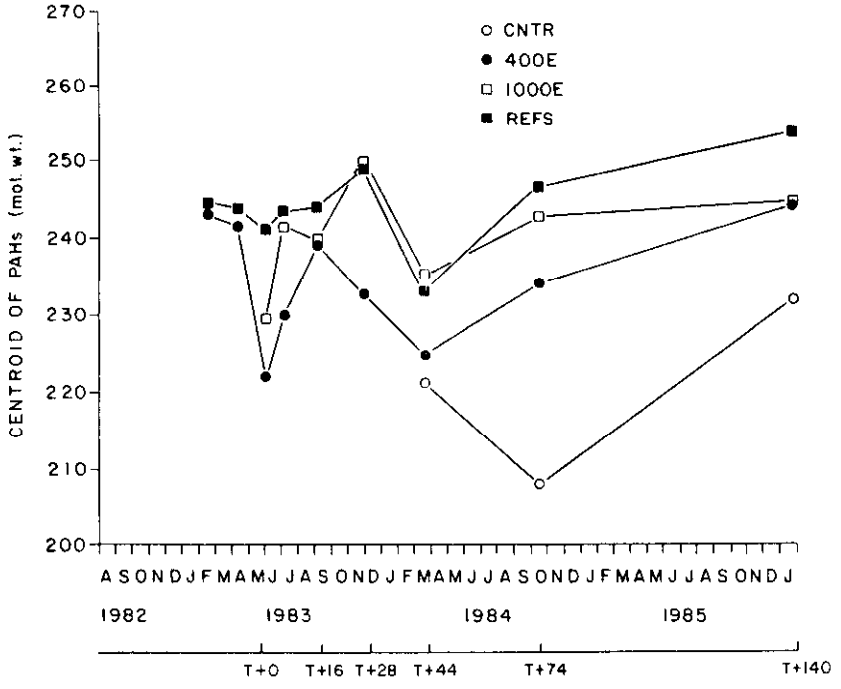


b. Benzo(a)pyrene

Figure 35. Concentrations of fluoranthene and benzo(a)pyrene in the tissues of *N. incisa* collected at the specified FVP stations and sampling dates

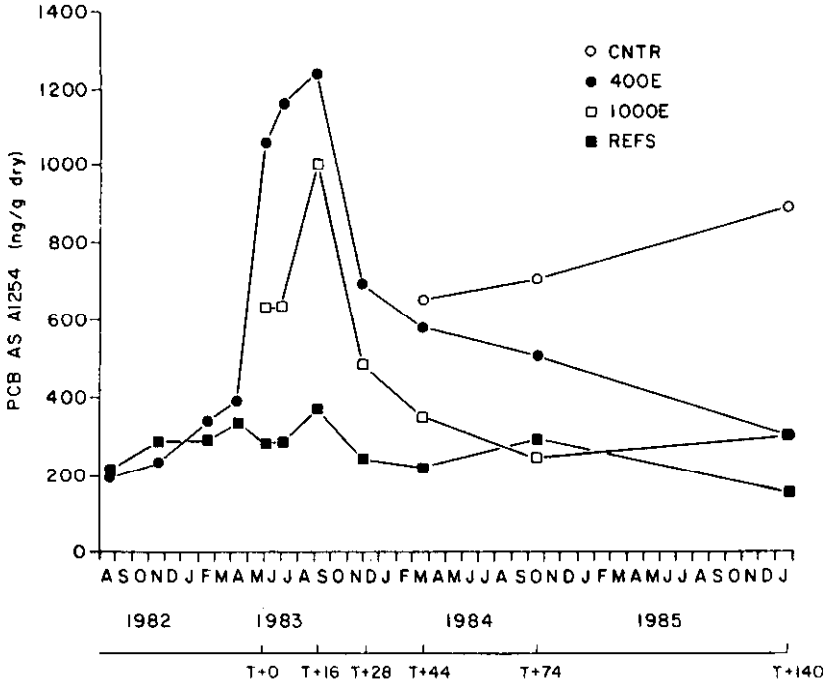


a. SUM of PAHs

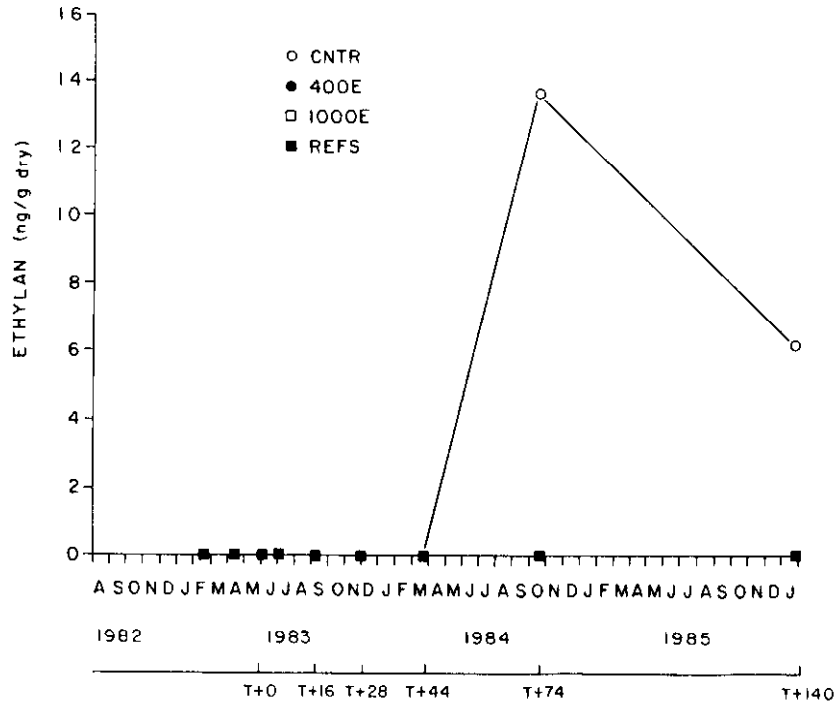


b. CENT of PAHs

Figure 36. Concentrations of the SUM of PAHs and CENT of PAHs in the tissues of *N. incisa* collected at the specified FVP stations and sampling dates

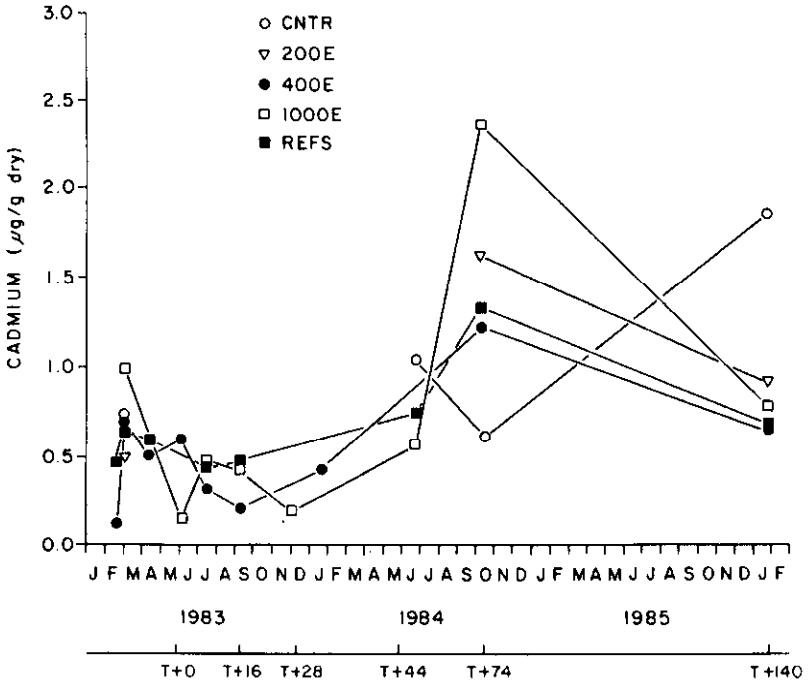


a. PCB as A1254

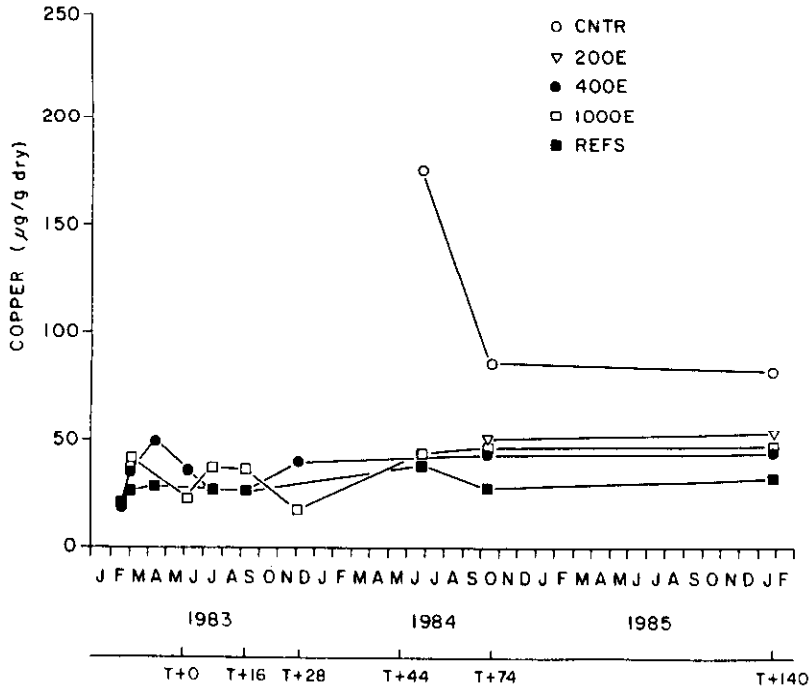


b. Ethylan

Figure 37. Concentrations of PCB as A1254 and ethylan in the tissues of *N. incisa* collected at the specified FVP stations and sampling dates. The value for CNTR on March 1984 (Figure 37b) was equal to REFS

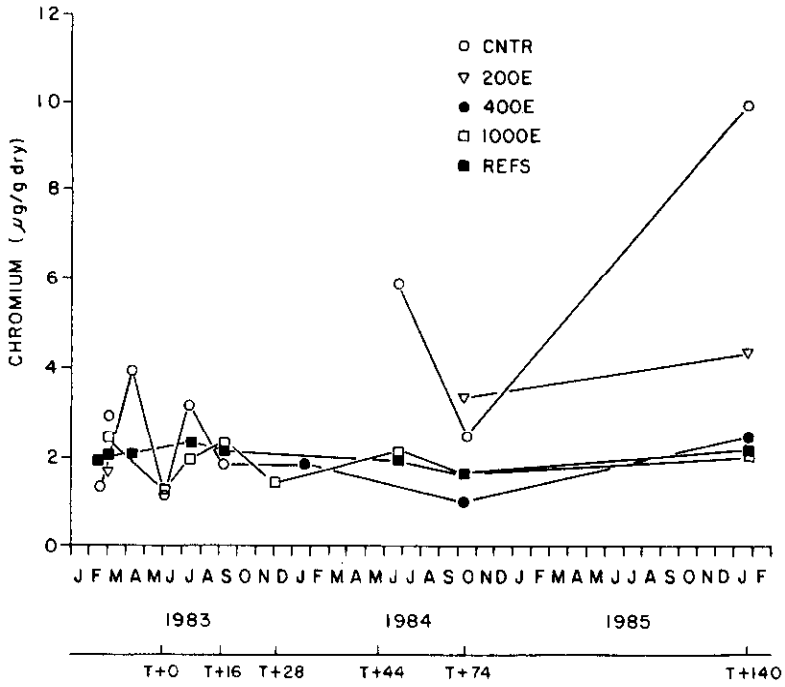


a. Cadmium

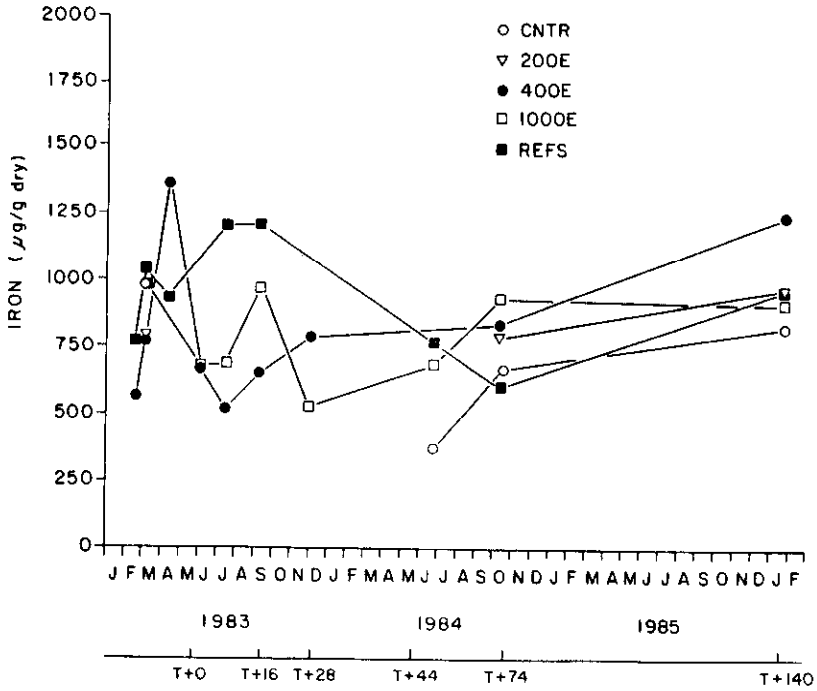


b. Copper

Figure 38. Concentrations of cadmium and copper in the tissues of *N. incisa* collected at the specified FVP stations and sampling dates



a. Chromium



b. Iron

Figure 39. Concentrations of chromium and iron in the tissues of *N. incisa* collected at the specified FVP stations and sampling dates

Effects results

122. *Mytilus edulis.* Adenine nucleotide concentrations were measured in adductor muscle tissue of *M. edulis* exposed at the FVP field stations. The results of these measurements are presented in Table 19. The data for adenine nucleotide pool concentrations and for AEC are presented graphically in Figure 40. The physiology of *M. edulis* is influenced by a variety of seasonal factors that varied naturally from collection to collection during the FVP. Because seasonal influences might conceal responses to the BRH sediments, the field exposure-effects data will be considered within discrete sampling periods, where mussels were presumably exposed to similar seasonal conditions. As was stated at the beginning of this report, the purpose for using adenine nucleotide responses was to determine whether relative sublethal effects could be measured between laboratory treatments and field stations. Consideration of field exposure-effects relationships within a sampling period is entirely consistent with this objective.

123. The use of field measurements of adenine nucleotide concentrations within a sampling period reduced sample size to a maximum of four when all stations were sampled. Regression analysis with this sample size is not appropriate; therefore, the data are presented graphically to illustrate trends. Because detailed field exposure data are not available, tissue concentrations are used to represent exposure concentrations experienced by the organisms. It would be impractical to present graphs for all of the residue-effects data for each of the 11 sampling dates. PCBs were selected because of the good relationship previously described between tissue concentrations of PCBs and BRH exposure concentrations in laboratory experiments. Therefore, only the residue data from the field for PCBs are used. These data are related to the effect responses of total adenylate nucleotide pool concentration and AEC. The total adenylate nucleotide pool concentration was the only adenylate nucleotide measurement that responded to BRH exposure in laboratory experiments. The AEC is included here because it is the primary response being evaluated in this report. When the data were examined in this manner, there were no trends among stations on any sampling dates for total adenine nucleotide pool concentrations or for AEC.

124. *Nephtys incisa.* Adenine nucleotide concentrations were measured in whole worms sampled at the FVP stations on the specified dates. The results of these measurements are presented in Table 20. The data for total

Table 19

Adenine Nucleotide Concentrations (μmol/g Wet Weight) Measured in Adductor Muscle Tissues of *M. edulis* Sampled at FVP Field Stations on Specific Dates

Station	ATP	ADP	AMP	Total	AEC
		22 Apr 83 (T - 04)			
CNTR	3.00	0.83	0.07	3.90	0.88
400E	3.06	0.83	0.11	4.00	0.87
1000E	2.74	0.79	0.09	3.62	0.86
REFS	2.94	0.80	0.05	3.79	0.88
		24 May 83 (T = 0)			
CNTR	--*	--	--	--	--
400E	--	--	--	--	--
1000E	3.69	0.98	0.12	4.79	0.87
REFS	3.40	0.85	0.13	4.38	0.87
		07 Jun 83 (T + 2)			
CNTR	--	--	--	--	--
400E	3.65	0.82	0.05	4.52	0.90
1000E	3.96	1.09	0.14	5.19	0.87
REFS	--	--	--	--	--
		13 Jul 83 (T + 8)			
CNTR	3.30	1.32	0.23	4.85	0.82
400E	3.59	1.28	0.19	5.06	0.84
1000E	3.40	1.19	0.24	4.83	0.83
REFS	2.84	1.20	0.32	4.36	0.83
		10 Aug 83 (T + 12)			
CNTR	3.71	1.47	0.39	5.57	0.80
400E	3.79	1.18	0.26	5.23	0.84
1000E	3.47	1.24	0.35	5.06	0.81
REFS	3.87	1.84	0.95	6.66	0.83

(Continued)

* Not sampled.

Table 19 (Concluded)

Station	ATP	ADP	AMP	Total	AEC
		06 Sep 83 (T + 15)			
CNTR	2.72	1.33	0.42	4.47	0.76
400E	2.61	1.54	0.50	4.65	0.73
1000E	2.79	1.49	0.43	4.71	0.75
REFS	2.31	1.22	0.42	3.95	0.74
		29 Nov 83 (T + 27)			
CNTR	--*	--	--	--	--
400E	3.27	1.07	0.13	4.47	0.85
1000E	4.06	0.93	0.06	5.05	0.90
REFS	3.86	1.08	0.09	5.03	0.88
		20 Mar 84 (T + 43)			
CNTR	2.47	0.88	0.06	3.41	0.85
400E	2.48	0.73	0.04	3.25	0.88
1000E	--	--	--	--	--
REFS	2.71	0.94	0.08	3.73	0.85
		05 Jun 84 (T + 55)			
CNTR	--	--	--	--	--
400E	3.84	0.89	0.10	4.83	0.89
1000E	--	--	--	--	--
REFS	3.52	0.91	0.11	4.54	0.88
		17 Oct 84 (T + 74)			
CNTR	--	--	--	--	--
400E	2.53	1.05	0.15	3.73	0.82
1000E	2.52	1.00	0.14	3.66	0.82
REFS	2.64	1.10	0.15	3.89	0.82
		14 Aug 85 (T + 116)			
CNTR	3.34	1.17	0.17	4.68	0.83
400E	3.23	1.17	0.22	4.62	0.82
1000E	2.90	0.94	0.14	3.98	0.84
REFS	3.95	1.15	0.08	5.18	0.87

* Not sampled.

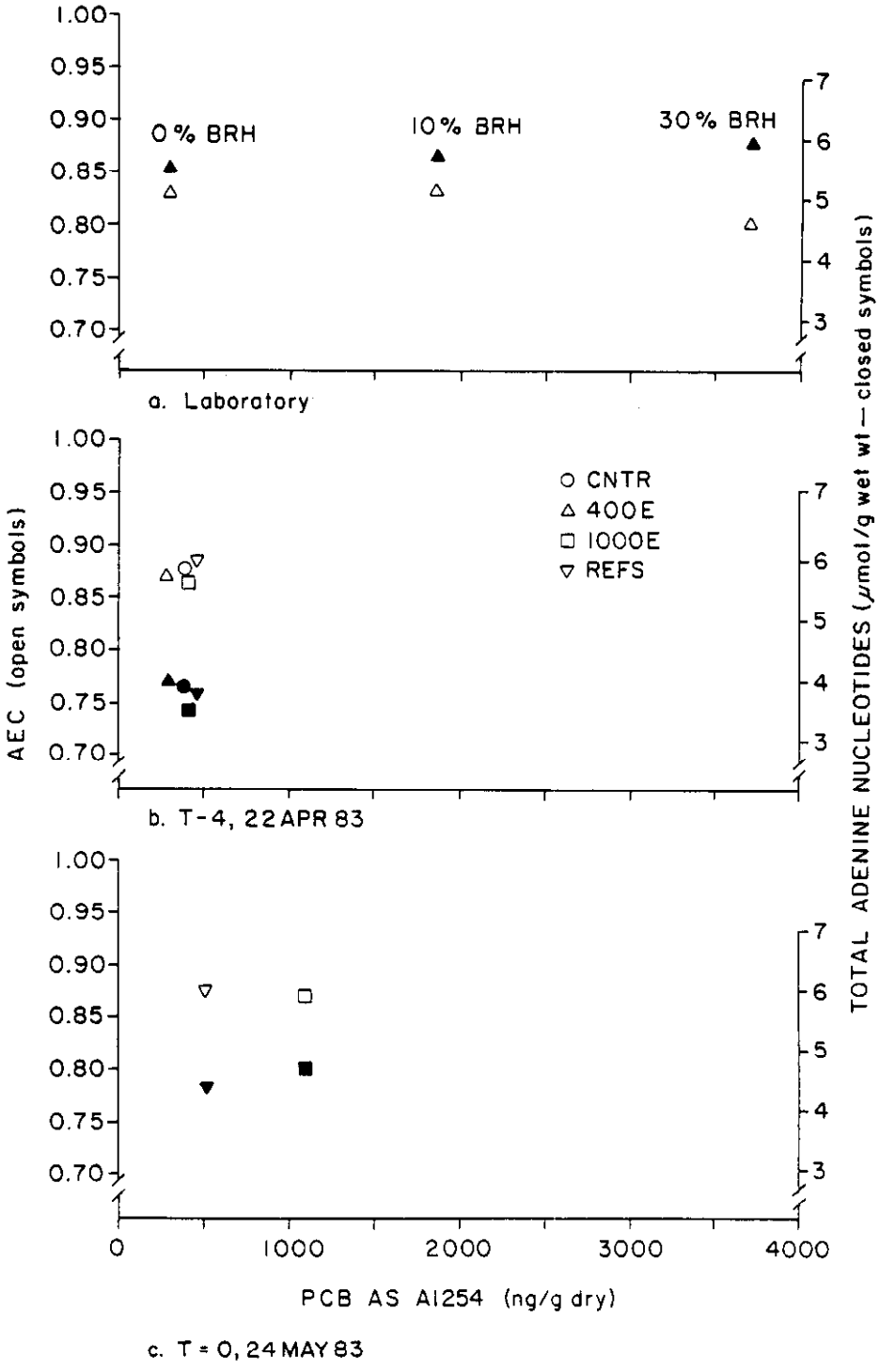


Figure 40. Relationship between adenine nucleotide concentration and AEC in *M. edulis* and PCB tissue residue concentrations in laboratory- and field-exposed animals. The laboratory data are presented to provide a perspective between the residue concentrations of laboratory- and field-exposed mussels (Sheet 1 of 4)

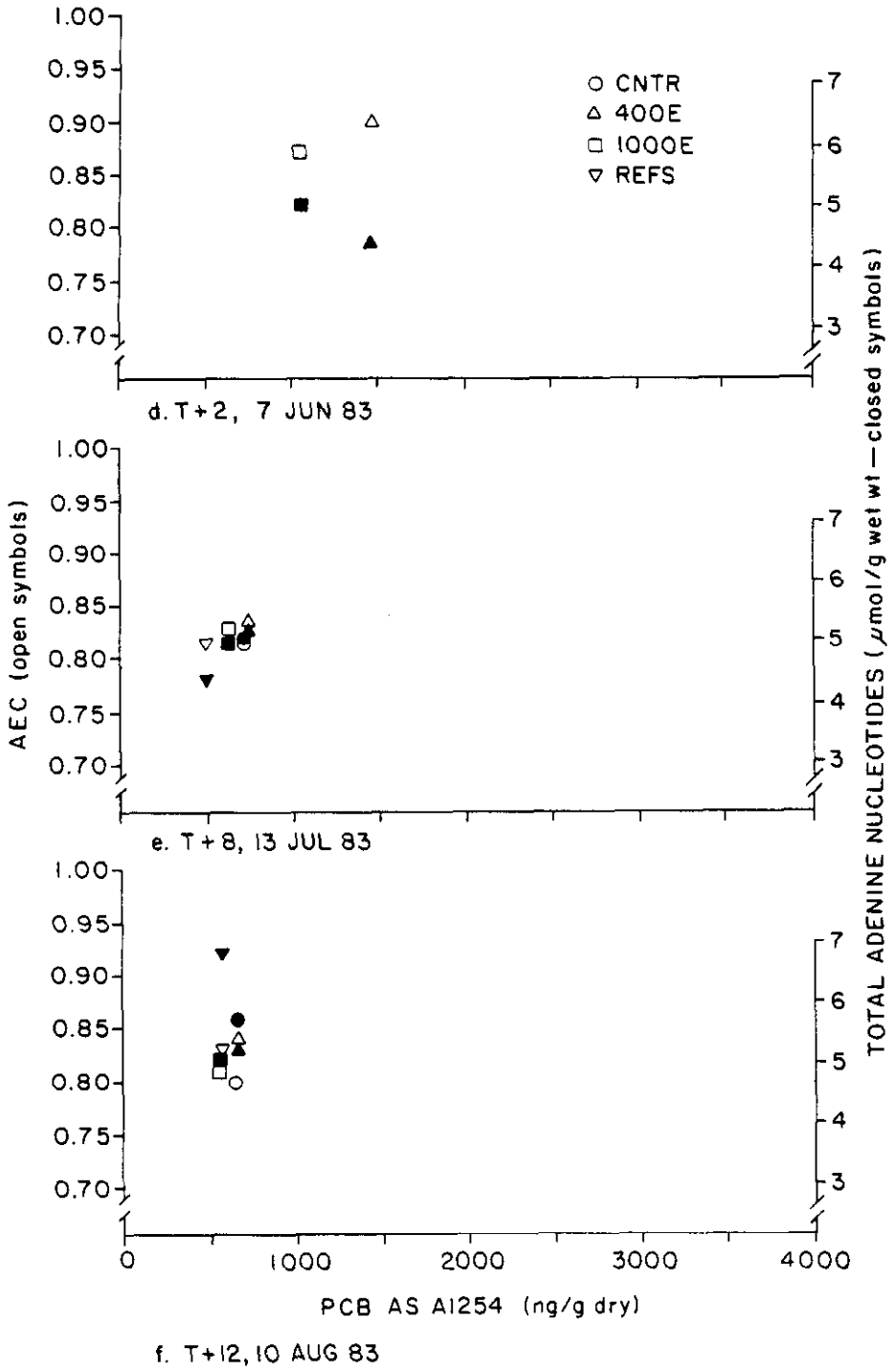


Figure 40. (Sheet 2 of 4)

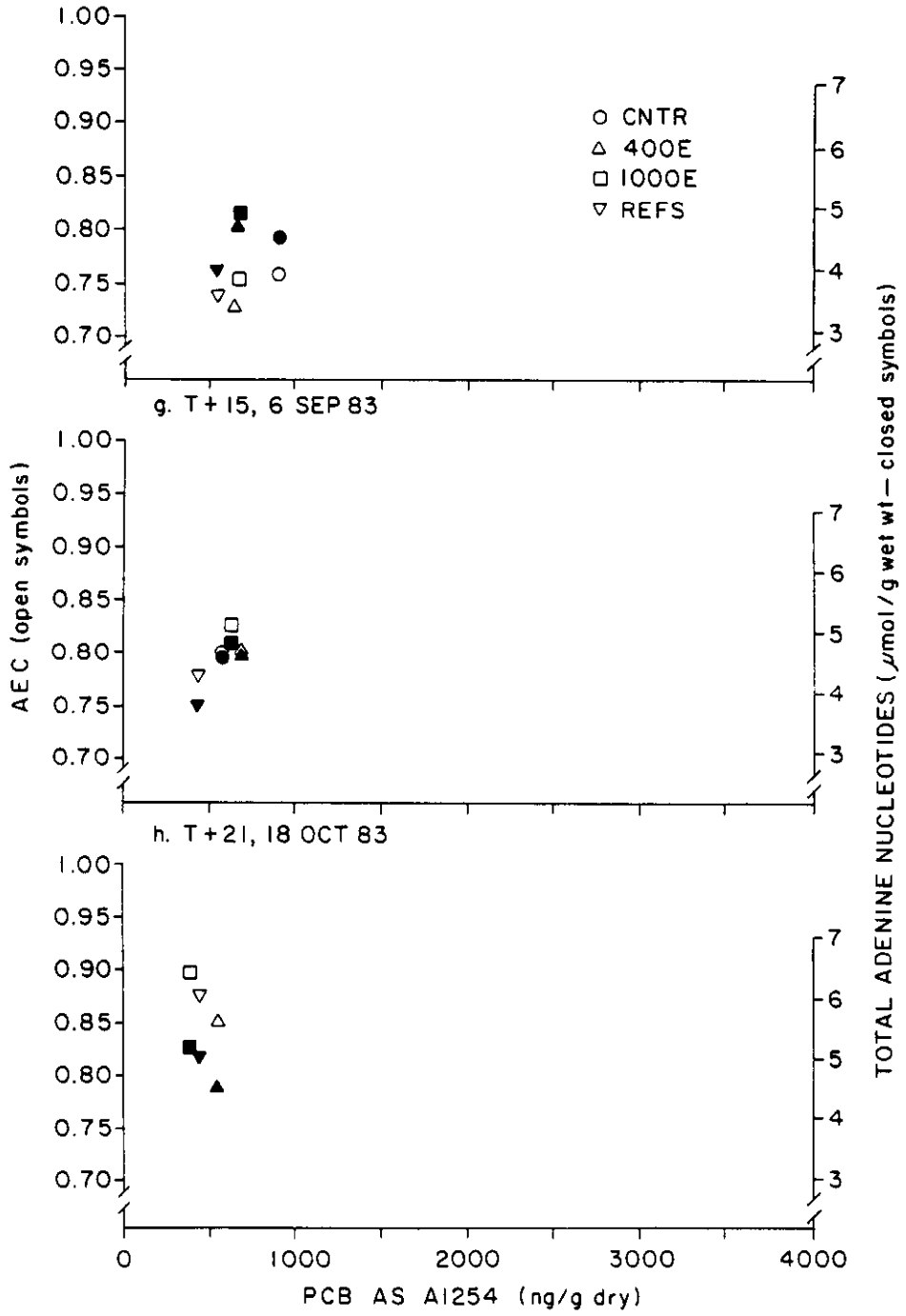


Figure 40. (Sheet 3 of 4)

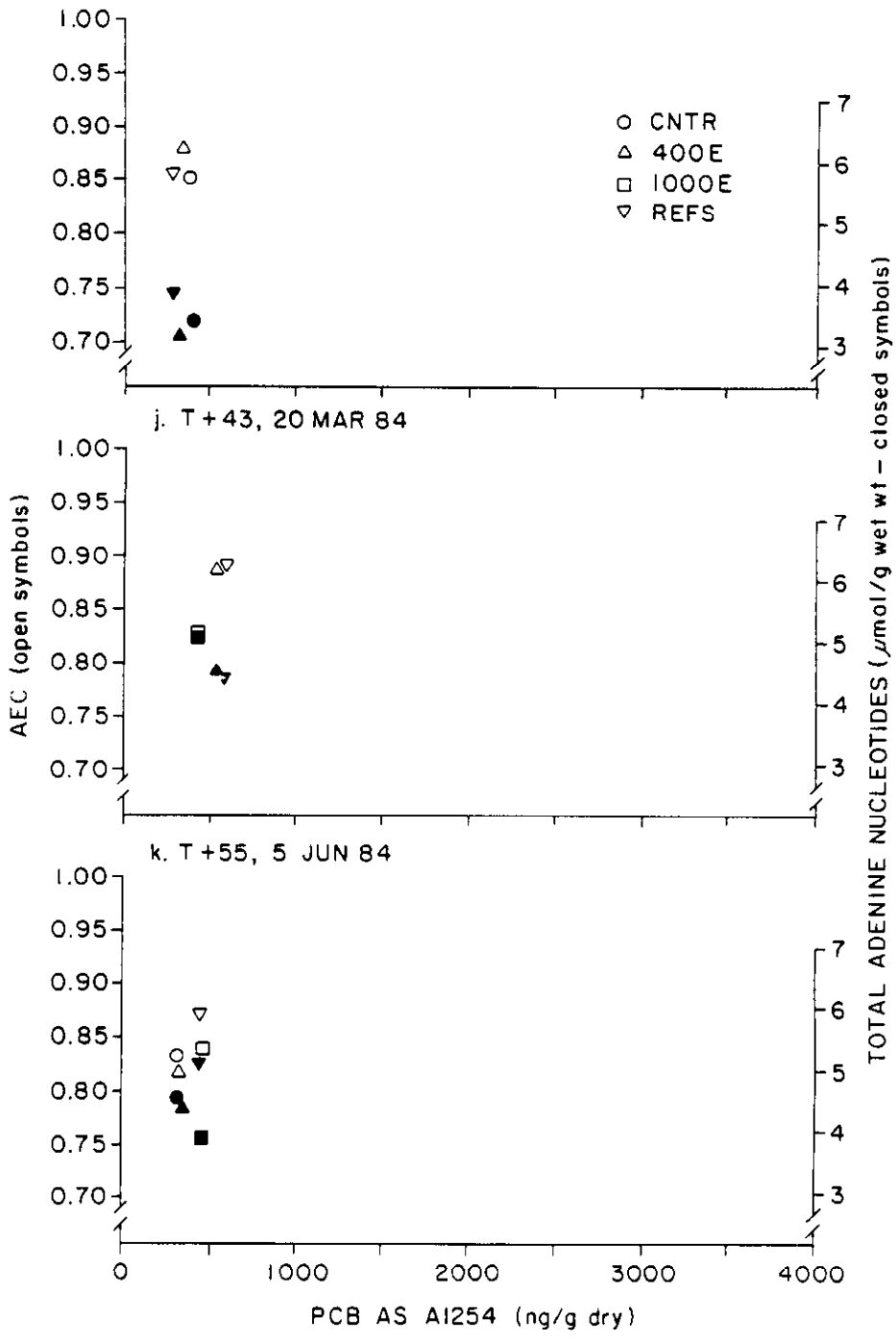


Figure 40. (Sheet 4 of 4)

Table 20

Adenine Nucleotide Concentrations (μmol/g Wet Weight) Measured in *N. incisa* Sampled on Specified Dates at the FVP Field Stations

Station	ATP	ADP	AMP	Total	AEC
		12 Apr 83 (T - 5)			
400E	0.61	0.16	0.05	0.82	0.36
1000E	--*	--	--	--	--
REFS	0.65	0.24	0.06	0.95	0.55
		06 Jun 83 (T + 3)			
400E	0.27	0.23	0.09	0.59	0.63
1000E	0.25	0.22	0.09	0.56	0.62
REFS	0.59	0.35	0.09	1.03	0.72
		19 Jul 83 (T + 9)			
400E	0.43	0.35	0.13	0.91	0.58
1000E	0.88	0.25	0.09	1.22	0.61
REFS	0.64	0.36	0.08	1.08	0.72
		08 Sep 83 (T + 16)			
400E	1.16	0.46	0.13	1.74	0.80
1000E	1.38	0.41	0.09	1.87	0.85
REFS	1.56	0.26	0.08	1.88	0.90
		14 Dec 83 (T + 30)			
400E	1.24	0.45	0.20	1.89	0.73
1000E	1.07	0.45	0.20	1.72	0.73
REFS	1.33	0.51	0.18	2.02	0.76
		15 Mar 84 (T + 43)			
400E	0.83	0.49	0.14	1.46	0.69
1000E	0.76	0.38	0.15	1.29	0.68
REFS	0.99	0.51	0.15	1.65	0.70

(Continued)

* Not sampled.

Table 20 (Concluded)

Station	ATP	ADP	AMP	Total	AEC
		21 Jun 84 (T + 57)			
400E	2.18	0.55	0.13	2.86	0.85
1000E	1.94	0.73	0.18	2.85	0.79
REFS	2.14	0.64	0.11	2.89	0.84
		10 Oct 84 (T + 73)			
400E	1.99	0.60	0.09	2.68	0.84
1000E	1.25	0.45	0.04	1.74	0.83
REFS	2.60	0.56	0.07	3.23	0.89
		26 Jun 85 (T + 110)			
400E	1.86	0.63	0.14	2.63	0.81
1000E	1.63	0.66	0.19	2.48	0.78
REFS	2.33	1.30	0.04	3.67	0.81

adenine nucleotide pool concentrations and for AEC are presented graphically in Figure 41. Because of seasonal influences on the organisms similar to those described for *M. edulis*, the data are compared among FVP stations only within sampling dates as was done for *M. edulis*. Limiting the comparisons of field measurements to within a sampling period reduced sample size to a maximum of three when all stations sampled for *N. incisa* were sampled. Regression analysis with this sample size is not appropriate; therefore, the data are presented graphically to illustrate trends. Because detailed field exposure data are not available, tissue concentrations of PCBs are used to represent BRH exposure concentrations experienced by the organisms. It would be impractical to present graphs for all of the data for each of the nine sampling dates. PCBs were selected because of the good relationship previously described between tissue concentrations of PCBs and BRH exposure concentrations in the laboratory experiment. The responses of total adenine nucleotide pool concentrations and AEC in *N. incisa* are presented for comparison with the data for *M. edulis* and because these responses represent two different ways of treating adenine nucleotide data. The pool is the simple sum of all the adenine nucleotide pools. The AEC is a weighted proportion of ATP in the total pool. Neither of these effects measurements responded to BRH exposure in laboratory experiments. When the field data were examined graphically, there were trends among stations for adenine nucleotide pool concentrations and for AEC on T + 16. There were no trends among stations on other dates.

Laboratory-to-Field Comparisons

Mytilus edulis

125. The laboratory-to-field comparison was completed in two parts and included both tissue residue and effects data. The approach taken was first to establish whether exposure conditions were similar in the laboratory and field residue data. Comparable tissue residues were interpreted as being indicative of comparable BRH exposures. The second step was to compare the adenine nucleotide pool concentrations and AEC values of the laboratory- and field-exposed mussels with similar tissue residue concentrations.

126. Residues. Results of the cluster analysis suggested several general observations. First, the samples that were most similar included all the field residues collected after T + 2 and the laboratory 0-percent BRH

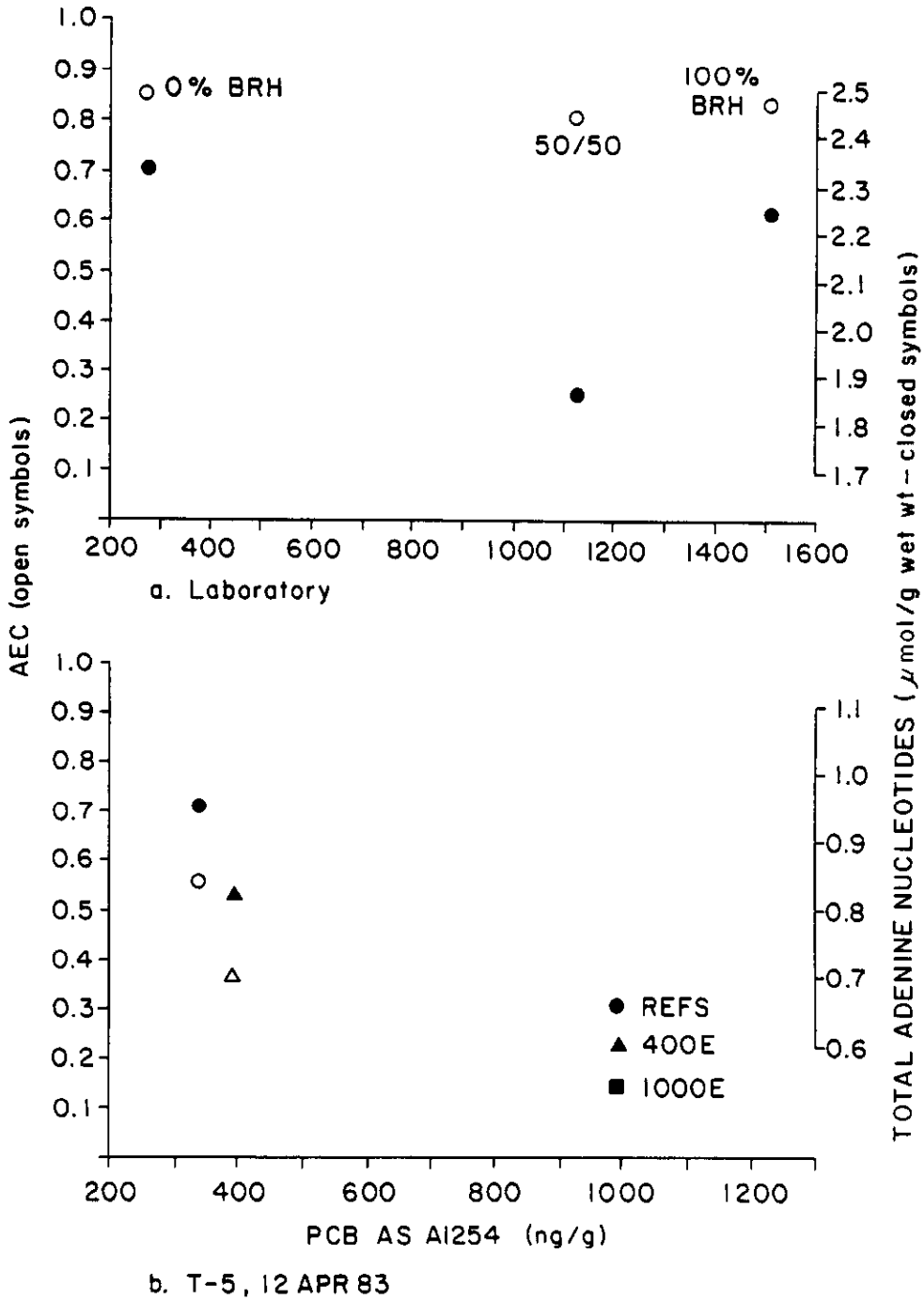


Figure 41. Relationship between total adenine nucleotide concentration and AEC in *N. incisa* and PCB tissue residue concentrations in laboratory- and field-exposed animals. The laboratory data are presented to provide a perspective between the residue concentrations of laboratory- and field-exposed worms (Sheet 1 of 4)

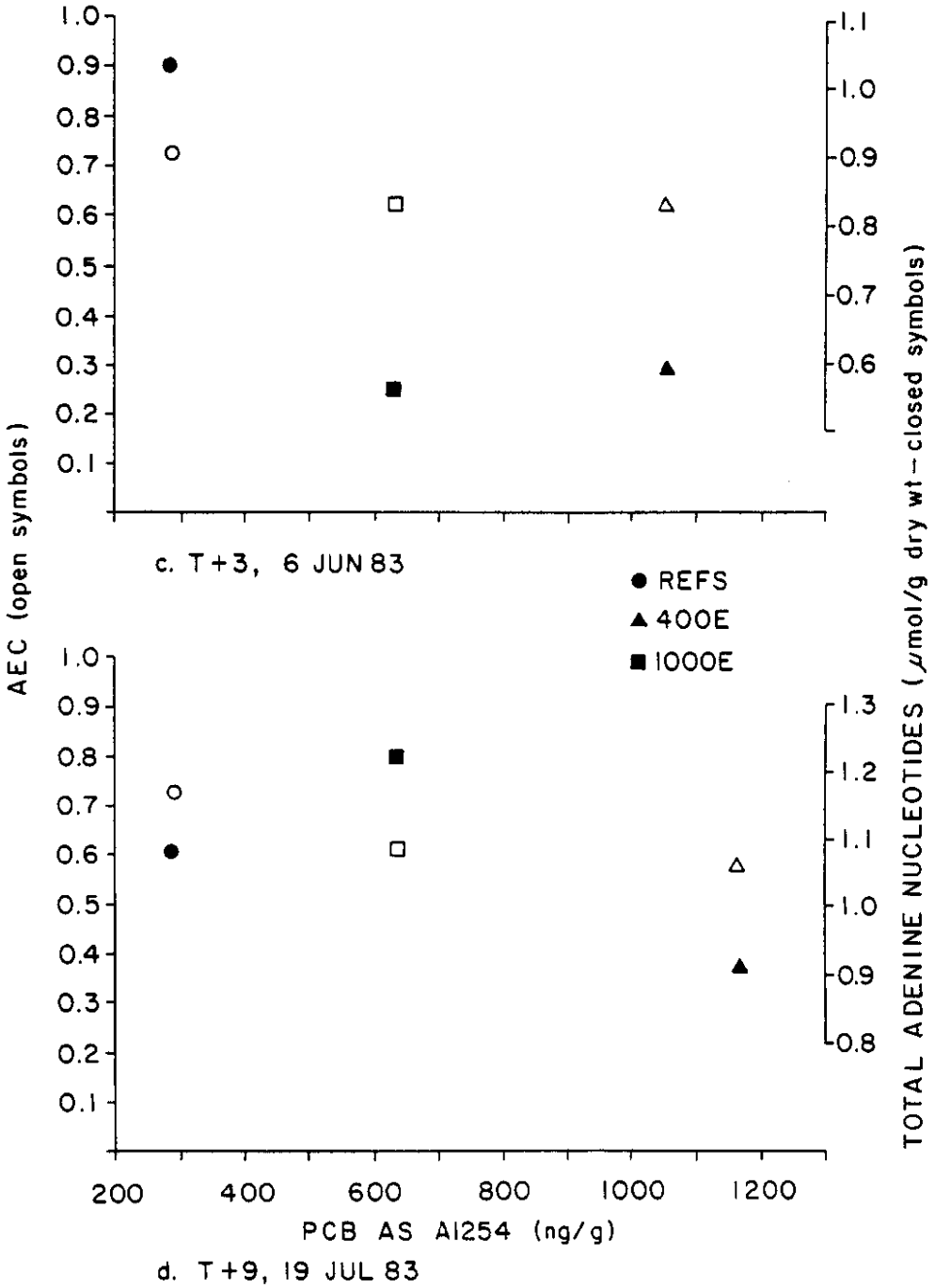


Figure 41. (Sheet 2 of 4)

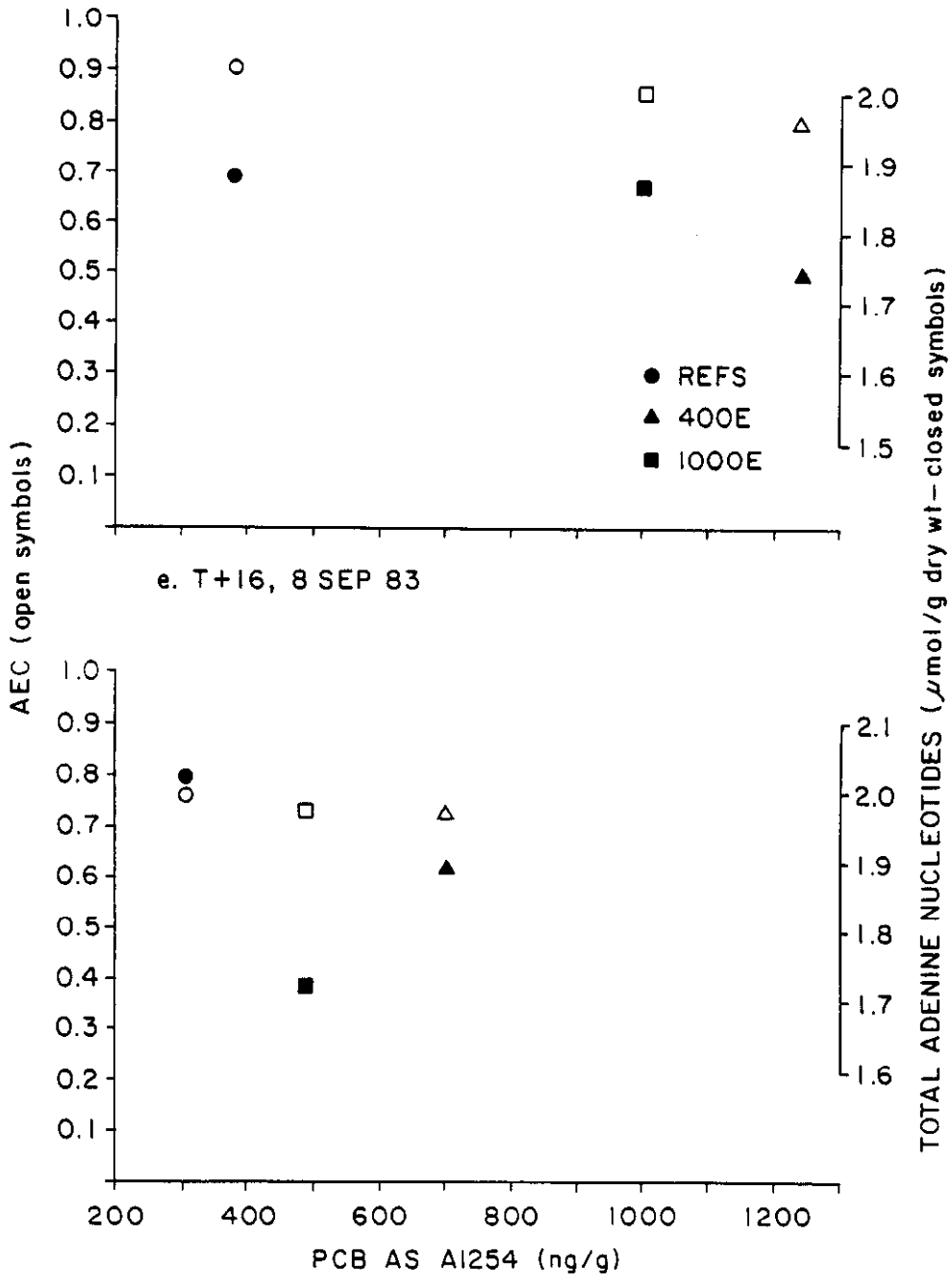


Figure 41. (Sheet 3 of 4)

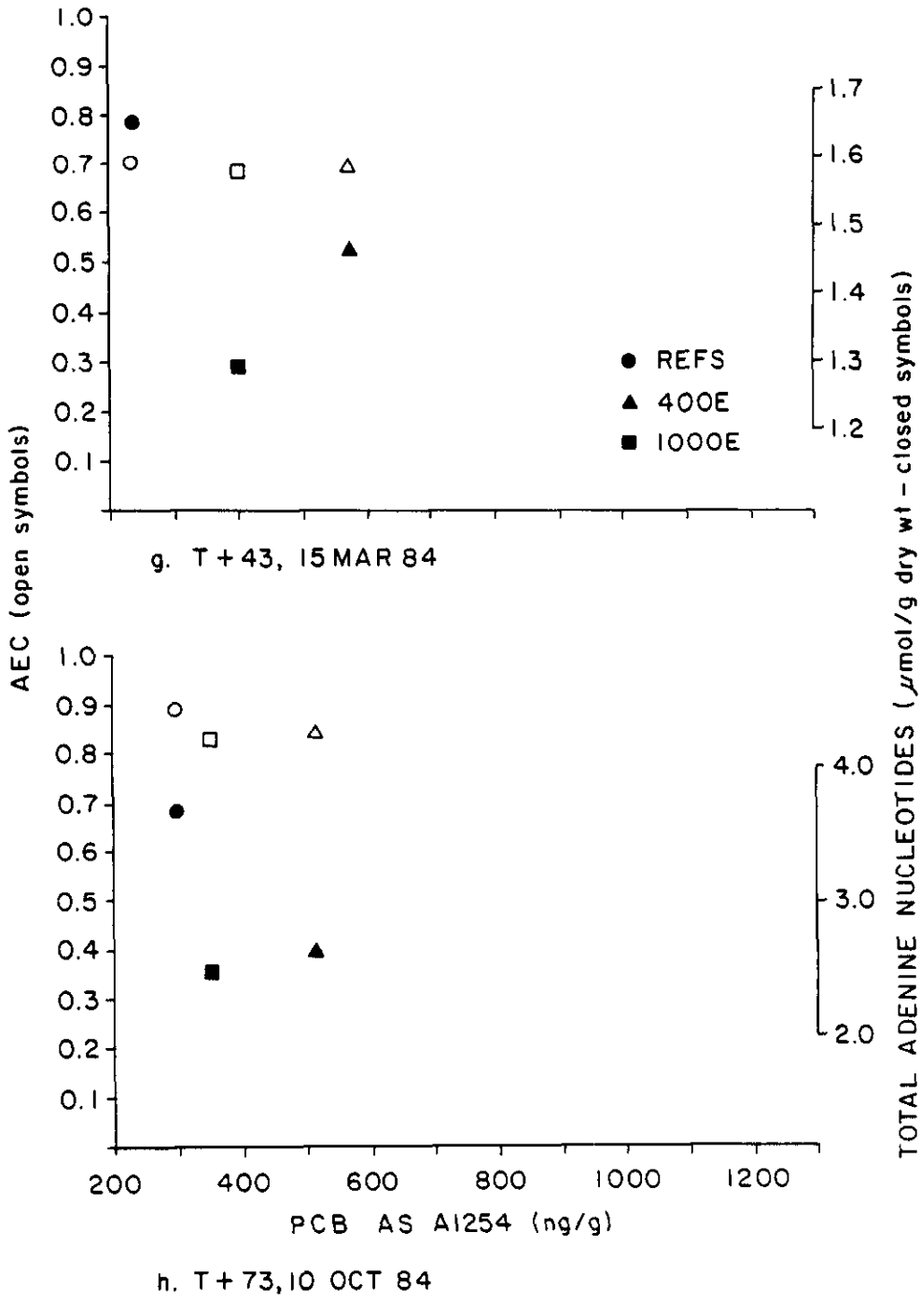


Figure 41. (Sheet 4 of 4)

exposures. This would indicate that mussels in the field received minimal exposure to BRH material after the initial disposal operation. Second, mussels collected predisposal (CNTR, 400E, 1000E) and those collected shortly after disposal (1000E at T + 0 and T + 2) were more similar to the other field samples than the laboratory samples. This would imply that, even during disposal, BRH exposures at these stations were more similar to subsequent postdisposal field residues than to laboratory BRH exposures. Third, all of the laboratory residues were more similar to each other than any of the field samples. This grouping would indicate that all laboratory exposures were very different from field exposures. Finally, mussel residues obtained from 400E at T + 2 were more similar to those of the laboratory-exposed mussels than the other field exposures. This sample was the last to cluster, indicating that it was not very similar to any other samples; however, it was more closely related to the laboratory samples than the field.

127. Effects. Analysis of the residue data suggested that the most valid comparison between laboratory and field adenylate nucleotide data would be between field samples, with the exception of 400E at T + 2 and laboratory mussels exposed to 0-percent BRH. Comparison of the adenine nucleotide data of mussels exposed to even 10-percent BRH in the laboratory to any mussels in the field would not be appropriate because the residues were dissimilar. Additionally, comparison of mussels collected from the field when environmental conditions were not similar to those in the laboratory would not be appropriate.

128. Laboratory experiments were conducted in the spring at water temperatures of 15° C. Comparable field conditions existed at T + 0 and T + 2 in the field. The AEC for mussels exposed to 0-percent BRH in the laboratory for 28 days was 0.83. The field-exposed mussels had AEC values of 0.87, 0.87, and 0.87 for the T + 0 (1000E, REFS) and T + 2 (1000E) collections, respectively. These AEC values were indicative of metabolically active individuals in a nonlimiting environment. The only other collection that occurred in the spring when water temperatures were similar was at T + 55. The AEC values for these mussels were 0.89 and 0.88 for stations 400E and REFS, respectively. These values are comparable with that value (0.83) for mussels exposed 28 days to 0-percent BRH in the laboratory. A comparison of the total adenylate nucleotide pool data for these same samples also reveals no clear differences between laboratory and field data.

129. Several generalizations are apparent from the comparison of laboratory and field results. The laboratory exposures indicated a good relationship between BRH exposure and residue concentrations in *M. edulis*. These data provide justification for assuming that lower residue concentrations in the field-exposed mussels were indicative of lower BRH sediment exposures in the field. In fact, the highest field residues were less than the residues of the mussels exposed to the lowest BRH sediment concentration (1.5 mg/ℓ) in the laboratory. Therefore, the data suggest that there was little or no overlap in laboratory and field BRH sediment exposure concentrations. The resultant effects data indicated adverse effects on mussels due to laboratory exposure, whereas in the field there were no effects attributable to BRH sediment exposure.

Nephtys incisa

130. The laboratory-to-field comparison was completed in two parts and included both tissue residue and effects data. The approach taken was to establish first whether exposure conditions were similar in the laboratory and the field by comparing laboratory and field residue data. Comparable tissue residues were interpreted as being indicative of comparable BRH exposures. The second step was to compare the adenine nucleotide pool concentrations and AEC values of the laboratory and field worms at similar exposures.

131. Residues. Data for the 12 representative chemical variables were analyzed statistically by cluster analysis. The purpose of this procedure was to identify distinctive patterns of association among the *N. incisa* sampled from laboratory experiments and field stations. The cluster analysis revealed no consistent clustering of the laboratory data separate from field data. This agrees with the overlapping range of residue data in laboratory and field samples. The implication is that laboratory exposures to BRH material accurately reflected the range of field exposures to BRH material for *N. incisa*.

132. Effects. The tissue residue data indicated that comparison of laboratory and field adenylate nucleotide data is valid. This comparison is appropriate when environmental conditions in the field are similar to those in the laboratory. Laboratory experiments were conducted with 20° C seawater. Comparable conditions existed at T + 8, T + 16, and T + 72 in the field. The AEC for worms exposed to 0-percent BRH sediment in the laboratory for 42 days was 0.85. The field-exposed worms had AEC values of 0.90, 0.85, and 0.80 for T + 16 (REFS, 1000E, and 400E, respectively) and 0.89, 0.83, and 0.84 for

T + 72 (REFS, 1000E, and 400E, respectively). The AEC values were very similar and were indicative of metabolically active individuals in a nonlimiting environment. The AEC values for worms from the T + 8 collection could not be used for comparison with other AEC values because of the extraction problems experienced at this time. A comparison of total adenine pool data for these same samples also revealed no clear differences between laboratory and field worms.

Residue-Effects Comparisons

133. Regression analysis was used to determine whether any relationship existed between the five biological measures (AEC, concentrations of ATP, ADP, AMP, and total adenine nucleotide pools) and the tissue concentrations of the selected 10 chemicals and 2 summary statistics for PAHs. The 5 biological measures were regressed on the 12 chemical variables for the laboratory and field data for both *M. edulis* and *N. incisa*. This results in a total of 240 regression analyses. It is not reasonable to present all of this information graphically in this report. Instead, these results are presented in Tables 21-24. These tables contain statistical P values indicating degree of statistical significance for each analysis. A P value of 0.05 or less indicates a statistically significant relationship between the two variables regressed together; a P value of 0.1 or less is useful for identifying possible trends in the data. The P values in Tables 21-24 were classified into significance categories of 0.05 or 0.1, by biological variable, by species, and by laboratory and field. This information is presented in Table 25. This table facilitates comparison of the regression analysis results in three ways: by biological variable, by species, and by laboratory versus field categories. For example, the results in the grand total rows at the bottom of the table permit ranking the biological variables from most to least correlated with tissue concentrations of the 12 chemical variables as follows: adenine nucleotide pool > total ATP > total ADP > AEC > total AMP. The results in the grand total column facilitate comparison by species and by laboratory versus field categories. For example, *M. edulis* had a total of 35 significant correlations ($P \leq 0.05$) out of a possible 120, while *N. incisa* had a total of 14 significant correlations out of a possible 120. Clearly, the biological responses of *M. edulis* were more closely correlated with tissue concentrations

Table 21

Summary of P Values Indicating Degree of Statistical Significance for Each Regression Analysis Between Biological Variables and Tissue Concentrations of Contaminants for Laboratory Samples of *M. edulis*

	Biological Variable				
Chemical Compound	ATP	ADP	AMP	Total Adenine Nucleotide Pool	AEC
Phenanthrene	0.282	0.424	0.608	0.063	0.405
Sum of 178 alkyl homologs	0.007	0.590	0.323	0.006	0.298
Fluoranthene	0.007	0.429	0.314	0.005	0.314
Benzo(a)pyrene	0.030	0.571	0.670	0.001	0.688
SUM of PAHs	0.008	0.459	0.416	0.003	0.383
CENT of PAHs	0.616	0.689	0.485	0.655	0.688
PCB as A1254	0.008	0.669	0.272	0.019	0.235
Ethylan	0.010	0.670	0.269	0.023	0.234
Copper	0.012	0.477	0.472	0.006	0.393
Cadmium	0.152	0.232	0.635	0.001	0.681
Chromium	0.684	0.085	0.160	0.054	0.235
Iron	0.681	0.688	0.516	0.304	0.615

of the 12 chemical variables than was true for the biological responses of *N. incisa*. A laboratory-versus-field comparison of the results revealed that *M. edulis* had 15 significant correlations in the laboratory versus 20 in the field, while *N. incisa* had 13 in the laboratory versus 1 in the field. With $P = 0.05$, out of 60 possible correlations, 3 can be expected by random chance alone. For *M. edulis*, slightly more correlations occurred in the field than in the laboratory. For *N. incisa*, the correlations were limited almost entirely to the laboratory.

134. These data may be examined also by chemical class. Table 26 presents the results of PAHs and metals by species and by laboratory versus field

Table 22

Summary of P Values Indicating Degree of Statistical Significance for Each Regression Analysis Between Biological Variables and Tissue Concentrations for Field Samples of *M. edulis*

	Biological Variable				
Chemical Compound	ATP	ADP	AMP	Total Adenine Nucleotide Pool	AEC
Phenanthrene	0.564	0.008	0.071	0.054	0.034
Sum of 178 alkyl homologs	0.117	0.147	0.171	0.684	0.062
Fluoranthene	0.309	0.012	0.035	0.495	0.009
Benzo(a)pyrene	0.072	0.496	0.363	0.434	0.199
SUM of PAHs	0.087	0.229	0.201	0.641	0.085
CENT of PAHs	0.093	0.001	0.034	0.002	0.036
PCB as A1254	0.044	0.314	0.486	0.036	0.669
Ethylan	0.076	0.382	0.298	0.508	0.142
Copper	0.305	0.011	0.004	0.323	0.007
Cadmium	0.447	0.499	0.134	0.459	0.491
Chromium	0.162	0.007	0.004	0.460	0.005
Iron	0.401	0.014	0.018	0.344	0.018

categories. For *M. edulis*, the results indicate correlations between adenylate nucleotide concentrations and both PAH and metal concentrations in both laboratory and field. For PAHs, there were eight significant correlations in the laboratory and five in the field. For metals, there were three in the laboratory and nine in the field. Overall, for *M. edulis*, the biological response correlations with PAHs (13) were about the same as with metals (12). For *N. incisa*, the correlations between biological responses and contaminant concentrations in tissues were limited almost entirely to PAHs in the laboratory (12 of 13).

Table 23

Summary of P Values Indicating Degree of Statistical Significance for Each Regression Analysis Between Biological Variables and Tissue Concentrations of Contaminants for Laboratory Samples of *N. incisa*

	Biological Variable				
Chemical Compound	ATP	ADP	AMP	Total Adenine Nucleotide Pool	AEC
Phenanthrene	0.006	0.042	0.626	0.002	0.050
Sum of 178 alkyl homologs	0.059	0.056	0.490	0.032	0.196
Fluoranthene	0.102	0.066	0.480	0.059	0.281
Benzo(a)pyrene	0.001	0.044	0.691	0.0002	0.026
SUM of PAHs	0.024	0.049	0.514	0.012	0.119
CENT of PAHs	0.297	0.151	0.673	0.217	0.491
PCB as A1254	0.135	0.045	0.733	0.078	0.392
Ethylan	0.124	0.078	0.473	0.075	0.280
Copper	0.256	0.089	0.460	0.165	0.513
Cadmium	0.174	0.151	0.274	0.118	0.362
Chromium	0.978	0.314	0.920	0.822	0.519
Iron	0.965	0.214	0.703	0.788	0.482

Table 24

Summary of P Values Indicating Degree of Statistical Significance for Each Regression Analysis Between Biological Variables and Tissue Concentrations for Field Samples of *N. incisa*

	Biological Variable				
Chemical Compound	ATP	ADP	AMP	Total Adenine Nucleotide Pool	AEC
Phenanthrene	0.053	0.181	0.930	0.057	0.417
Sum of 178 alkyl homologs	0.060	0.328	0.710	0.078	0.540
Fluoranthene	0.068	0.293	0.830	0.081	0.513
Benzo(a)pyrene	0.134	0.617	0.529	0.185	0.919
SUM of PAHs	0.078	0.378	0.651	0.102	0.639
CENT of PAHs	0.056	0.663	0.651	0.095	0.374
PCB as A1254	0.338	0.609	0.719	0.373	0.753
Ethylan*	--	--	--	--	--
Copper	0.720	0.955	0.200	0.812	0.501
Cadmium	0.074	0.115	0.076	0.091	0.292
Chromium	0.328	0.111	0.481	0.244	0.081
Iron	0.905	0.194	0.032	0.654	0.328

* Ethylan could not be quantified because of analytical interference.

Table 25

Summary of the Number of Significant Correlations Between Adenine Nucleotide Concentrations and Tissue Contaminant Concentrations by Biological Variable, by Species, and by Laboratory Versus Field Categories

Species	Lab/ Field	Signif- icance	Biological Variable ATP	ADP	AMP	Total Adenine Nucleotide Pool	AEC	Grand Total
M. edulis	Lab	0.05	7	0	0	8	0	15
		0.10	7	1	0	10	0	18
	Field	0.05	1	6	5	2	6	20
		0.10	5	6	6	3	8	28
	Total	0.05	8	6	5	10	6	35
		0.10	12	7	6	13	8	46
N. incisa	Lab	0.05	3	4	0	4	2	13
		0.10	4	8	0	7	2	21
	Field	0.05	0	0	1	0	0	1
		0.10	6	0	2	5	1	14
	Total	0.05	3	4	1	4	2	14
		0.10	10	8	2	12	3	35
Grand Total		0.05	11	10	6	14	8	49
		0.10	22	15	8	25	11	81

Table 26

Summary of the Number of Significant Correlations Between Adenine Nucleotide Concentrations and Tissue Contaminant Concentrations by Chemical Class, by Species, and by Laboratory Versus Field Categories

Species	Lab/ Field	Significance	PAHs*	Metals*
M. edulis	Lab	0.05	8	3
		0.10	9	5
	Field	0.05	5	9
		0.10	11	9
	Total	0.05	13	12
		0.10	20	14
N. incisa	Lab	0.05	12	0
		0.10	16	1
	Field	0.05	0	1
		0.10	7	5
	Total	0.05	12	1
		0.10	23	6

* Maximum possible number of significant correlations for PAHs = 25, for metals = 20 for species, for laboratory or field category.

PART IV: DISCUSSION

135. The objectives of this study were to: (a) field verify laboratory results and (b) investigate residue-effect relationships in *M. edulis* and *N. incisa* after exposure to BRH sediment in the laboratory and in the field. The design of this study followed a logical progression from BRH exposure to tissue residue concentration to biological effects. The discussion will parallel this approach by establishing the exposure-residue and residue-effect relationships separately for the laboratory and for the field. This permits a comparison of the laboratory and field results. Finally, the residue-effects relationship will be considered in depth.

Laboratory Experiments

Mytilus edulis

136. There was a strong link between exposure to BRH sediment and selected tissue residues in *M. edulis*, as confirmed by the monitoring data collected during the laboratory experiments. In addition, the relationship between mussel tissue residues for several chemical contaminants demonstrated that compounds with higher molecular weights and stability, PCBs in particular, tracked the BRH exposure concentrations remarkably well. For example, tissue residue data indicated that mussels from the 30-percent BRH chamber exhibited twice the level of PCBs as those in the 10-percent BRH chamber. Corresponding monitoring data indicated that the actual delivered level of BRH sediment was 3.3 and 1.5 mg/ℓ, respectively, for those two chambers, indicating that PCBs were a good "marker" for exposure to BRH material. Because of this direct relationship, residue concentrations can be assumed to be indicative of exposure concentration for highly stable compounds such as PCB. This relationship is particularly important in the field where direct, continuous monitoring data of exposure conditions are difficult, if not impossible, to collect.

137. The strong exposure-residue relationships measured in the laboratory experiments indicate beyond reasonable doubt that the contaminants in BRH sediments are biologically available. That mussels were affected by exposure to BRH sediments is evident by reduced scope for growth, clearance (feeding) rate, and shell growth rate in both experiments (Nelson et al. 1987).

138. In this report the responses of adenine nucleotides and AEC to stress are considered. The central role of adenine nucleotides in energy transformation and in metabolic regulation suggests their potential usefulness as indicators of sublethal stress (Vetter and Hodson 1984). The adenine nucleotides were measured in the adductor muscle tissue of *M. edulis* from all treatments in laboratory Experiments 1 and 2. The exposed organisms from these experiments were stressed as evidenced by reduced scope for growth including reduced clearance (feeding) rates (Nelson et al. 1987). It might be expected that under stress conditions and reduced food intake, the adenine nucleotide pools of these organisms should be affected. Of the five variables considered, ATP, ADP, AMP, adenine nucleotide pool, and AEC, only the pool responded. This response was significant ($P = 0.001$, $r^2 = 0.94$) only for the day 14 data pooled from both experiments. The AEC measured in *M. edulis* did not respond to BRH exposure in the laboratory.

139. Caution should be exercised when interpreting adenine nucleotide concentration data as indicators of stress. These concentration data represent "standing crop" or "static" pool sizes and convey little or no information about energy flow. The turnover time for ATP in metabolically active tissues may be less than 1 sec, and its concentration relative to energy storage compounds is exceedingly low (Vetter and Hodson 1984). Therefore, ATP does not represent an energy reserve but, rather, the ability of the cell to generate energy. It is the rate of energy flow through ATP between anabolic and catabolic processes that is of interest in measuring an organism's response to stress. A stressor may affect metabolism by increasing the demand for energy and/or by inhibiting the production of energy. If a stress causes an increase in metabolism (and therefore energy flow) without exceeding the organism's ability to regenerate ATP from energy reserves, there will be no change in ATP concentration. Changes in ATP concentration will occur if a stress causes a metabolic demand that exceeds the organism's ability to regenerate ATP; if the energy reserves are exhausted; or if the stress blocks the ability to resynthesize ATP. AEC is a less sensitive indicator of stress because it may remain constant even when decreases in ATP result in decreases in total adenylate concentrations rather than increases in ADP and AMP. Vetter and Hodson (1984) state, "A change in total adenylate concentration is precisely what does occur to a greater or lesser extent in almost all organisms studied to date." The response of *M. edulis* in this study to BRH exposure in

the laboratory is consistent with this explanation. The total adenylate concentration in *M. edulis* muscle tissue decreased with exposure to increasing concentrations of BRH sediment, while the AEC remained unchanged. Atkinson (1977) proposed AEC as a biochemical concept and explained in great detail its conservative and cybernetic nature. These very qualities make AEC insensitive as an indicator of stress.

Nephtys incisa

140. The tissue residue concentrations of all the organic compounds measured in *N. incisa* increased with increasing exposure to BRH sediments. This strong exposure-residue relationship measured in the laboratory experiments indicates beyond reasonable doubt that the worms were exposed to BRH sediments and that the contaminants in BRH sediments were biologically available. That worms were affected by exposure to BRH sediments is evident by reductions in growth, increases in respiration rate, and exposure-related reductions in net growth efficiency (Johns and Gutjahr-Gobell 1988).

141. Adenine nucleotides were measured in whole worms from all treatments at all sampling times in three laboratory experiments. Exposure conditions ranged from time zero, nonexposed, to 200 mg BRH/ℓ for 42 days. Of the five variables considered, ATP, ADP, AMP, adenine nucleotide pool, and AEC, none exhibited a response to BRH exposure. Caution should be exercised when interpreting apparent "no-effect" responses in adenine nucleotide concentration data. These data represent static or snap-shot measurements of adenine nucleotide pool sizes and therefore convey little or no information about energy flow. Ideally, these measurements should be supported by measurements of energy storage compounds such as lipid and glycogen. Vetter and Hodson (1984) state:

> Adenylate measurements alone fail to pinpoint a specific cause and effect relationship between a type of pollutant and an observed effect. This is not a failure of adenylate measurements in particular but an unavoidable consequence of the generalized stress response as it has evolved in most organisms. Combining adenylate measurements with other measures of energy reserves, such as lipid and glycogen, can improve detection of low-level chronic stress and begin to indicate the mechanism of toxic action.

Field Experiments

Mytilus edulis

142. The exposure-residue relationships generated in the field portion of this study were not as straightforward as those described for the laboratory studies. While the laboratory experiments provided as constant a set of exposure conditions as possible, the data required for defining comparable exposure conditions in the field were difficult, if not impossible, to collect. Consequently, the exposure-residue relationships established in the field were, by necessity, more qualitative than quantitative.

143. Although qualitative, estimates of BRH exposure in the field were necessary to establish exposure conditions for the laboratory-to-field comparison (i.e., establish when exposure conditions were similar in the laboratory and in the field). The following generalizations are evident from the estimated BRH exposure concentrations: (a) the two independent methods, tissue residue and whole water analysis, provided remarkably similar estimates of BRH exposure; and (b) there was a distinct exposure signal at 1 m above the bottom during and immediately postdisposal, and that signal was transient, decreasing spatially and temporally postdisposal.

144. Comparison of the tissue residue and water chemistry estimates of BRH concentrations in CLIS indicated very good correspondence as demonstrated by the following examples. Tissue residue data (PCBs) from the T + 2 collection indicated that the BRH concentration was estimated to range between 1.4 and 0.8 mg/ℓ at the CNTR station. Water samples from the same station estimated the BRH concentration to range between 1.1 and 0.7 mg/ℓ using PCB values, and 1.3 and 0.7 using copper values. Approximately 1 month later, BRH concentration estimates, based on PCB tissue residues, were between 0.7 and 0.2 mg/ℓ at the CNTR station. The corresponding water chemistry estimates of BRH concentrations at the CNTR station ranged from 0.2 to 0.1 mg/ℓ, using PCBs, and 0.6 to 0.3 mg/ℓ, using copper. The similarity of these estimates indicates that exposure based on whole water chemistry concentrations and tissue residue concentrations tracked reasonably well.

145. The good relationship between residue and water chemistry estimates of BRH concentrations further supports the second generalization mentioned above, that, BRH exposure 1 m above the bottom was maximal immediately postdisposal and decreased over time. Spatially, both the PCB tissue residue

and water chemistry data indicated that BRH exposure decreased moving away from the CNTR station immediately postdisposal. This pattern persisted until T + 12, when tissue residues were similar at each station. The loss of the spatial differences in BRH exposures would suggest that exposure from the disposal mound was minimal after this collection period. Temporally, the maximum estimated concentration of BRH material 1 m above the bottom ranged between 1.4 and 0.8 mg/ℓ (tissue residues at T + 2, Table 15). At the time of the next collection, this value decreased by approximately one half and continued to decrease over time.

146. While a range was calculated to estimate BRH exposure concentrations, it is interesting to note the temporal pattern of the high and low estimates. The high estimate of BRH exposure never reached zero, even in the later collections (i.e., T + 55, T + 116). This may indicate that background PCB levels in CLIS contributed to the BRH estimates, including those immediately postdisposal. The low estimate, calculated by subtracting the concentration at the REFS station, was assumed to remove the background concentration present in CLIS. Therefore, the low estimate, while providing a measure of relative difference between the stations, also may have provided the best estimate of actual BRH concentration. However, even using the high estimates, the data suggest that the integrated exposure of BRH material to *M. edulis*, 1 m above the bottom, was minimal at all the FVP stations and decreased rapidly following completion of the disposal operation.

147. Two weeks postdisposal (T + 2), when maximum tissue residues (i.e., exposures) occurred, no decreases in AEC or any of the adenine nucleotide concentrations were observed. One explanation is that the BRH exposure concentration at T + 2 may have been insufficient to cause a response. The estimated maximum BRH exposure at a station where mussels were deployed (0.8 mg/ℓ was about half that of the lowest suspended sediment concentration tested in the laboratory (1.5 mg/ℓ). The response threshold for the adenine nucleotide was >5 mg/ℓ BRH suspended sediment. Therefore, the BRH signal in the field was "weaker" than that required to elicit a response in the laboratory experiments. Based upon laboratory exposure-response data, one would have predicted that for the measured field exposures no effects would occur to the adenine nucleotides. This is in fact exactly what was observed.

148. AEC and adenine nucleotide concentrations for mussels deployed in CLIS for 1 month and longer were affected only by natural phenomena. Mussels

collected during August and September, regardless of location, had AEC values indicative of stress. This phenomenon has been reported for mussels previously by Skjoldal and Barkati (1982) and Zaroogian et al. (1982) and is probably due to a combination of reproductive and temperature (22° C) stresses.

149. The final collection (T + 116), a 1-month deployment, indicated that AEC was not different among stations; however, the AEC values were higher than the AEC for these same mussels at the time of deployment. This was due to an abnormal occurrence that was endemic to Narragansett Bay, including the site of the reference population, at the time mussels were collected for deployment in CLIS. A bloom of a small (<2.0 μ) algal species occurred throughout Narragansett Bay. This alga, present in concentrations greater than 1.0 billion cells/ℓ, caused the mussels in the Bay to cease feeding, resulting in mass mortalities in mussel populations. As a result, mussels deployed for T + 116 were not in the best condition, and deployment in CLIS appeared to be therapeutic.

Nephtys incisa

150. Tissue concentrations of PCBs in *N. incisa* increased at all FVP stations during the summer of 1983 and reached their highest measured concentrations in September. This evidence indicates that exposure to dredged material at the sediment-water interface continued throughout the CLIS study area during the summer of 1983. Since there were no significant storms during the summer of 1983, the contaminant exposures were probably due to initial dispersion of dredged material and tidally driven resuspension and movement of sediments from the dredged material mounds.

151. There were difficulties with adenylate extraction from *N. incisa* during the first several months of field sampling. These problems were resolved by September 1983 (Zaroogian et al. 1985). Therefore, the data from September 1983 (T + 16) could be tested for spatial differences. Differences among stations were found to be significant for all adenine nucleotide concentrations and AEC. The differences in AEC were highly significant ($P = 0.0001$) with the lowest values closest to the disposal mound: 400E (0.80), 1000E (0.85), and REFS (0.90). Although these differences were statistically significant, the AEC values were indicative of nonstressed organisms.

152. During the winter months (T + 29, T + 43), what appeared to be a seasonal stress occurred. This stress appeared to be greater than any stress induced by disposal operations. Davis (1979) reported a sixfold to sevenfold

reduction in respiration in *N. incisa* during winter months (seawater temperature 0° C) when compared with respiration during the summer months (seawater temperature 24° C). Seawater temperatures recorded at the December 1983 and March 1984 samplings were 7° C and 0.9° C, respectively. Thus, the AEC values for *N. incisa* during winter months, which were indicative of metabolic stress, could have been due to lowered respiration rates caused by low seawater temperatures.

Laboratory-to-Field Comparisons

153. A primary objective of the FVP was to field verify the laboratory biological responses by measuring the same response under both laboratory and field exposures. A basic and often implicit assumption is that results derived from laboratory tests are directly applicable in the field. This study was designed to test that assumption.

Mytilus edulis

154. Exposure conditions must be examined to determine whether the biological responses are responding to comparable exposures in the laboratory and in the field. A comparison of laboratory and field data indicated one obvious fact: laboratory and field BRH exposures were different. The two independent estimates of BRH exposure indicated that maximum exposures in the field were half that of the lowest BRH exposure in the laboratory. Cluster analysis of laboratory and field mussel residue data yielded results similar to those obtained in estimating the field exposures. That is, the mussel residues in the field were most similar to mussels exposed to reference sediment in the laboratory. Considering these data, discussion of the laboratory-field comparison will focus initially on when conditions (exposures and residues) were similar in the laboratory and field, and secondly on estimated concentrations of BRH suspended material required to affect concentrations of adenine nucleotides and AEC values.

155. The exposure and residue data indicated that the most legitimate comparison between laboratory and field biological effects data was between all field samples (with the exception of 400E at T + 2) and laboratory mussels exposed to 0-percent BRH. Furthermore, because temperature and season during the T + 0, T + 2, and T + 55 collections were most similar to the laboratory exposures, these biological effects data should provide the most accurate

laboratory-to-field comparison. The AEC value for mussels exposed to 0-percent BRH sediment in the laboratory for 28 days was 0.83. The field-exposed mussels exhibited AEC values of 0.87, 0.87, and 0.87 for the T + 0 (1000E), T + 0 (REFS), and T + 2 (1000E) collections, respectively. The similarity of these AEC values indicated that the relative physiological condition of these mussels was the same when environmental conditions were most alike. The mussel collection at T + 55 also occurred in the spring at water temperatures similar to the laboratory exposures; however, these mussels had been deployed in CLIS for 8 months. The AEC values of these mussels, 0.87, 0.83, and 0.88 for 400E, 1000E, and REFS, respectively, were similar to the AEC for mussels in the 1-month laboratory exposure (0.83).

156. The purpose of this qualitative comparison between laboratory and field was to determine whether similar exposures produced similar results. In light of the differences between the actual laboratory exposures (constant exposure levels, food supply, etc.) and field exposures (fluctuating particulate concentrations, food quantity, etc.), the AEC values and the adenine nucleotide concentrations between the two exposures were remarkably similar.

157. A second aspect of the laboratory-to-field comparison was a qualitative evaluation of the estimate of BRH material required to produce an effect on AEC and the adenine nucleotide concentrations in the laboratory and field. The total adenine pool concentration for mussels in the laboratory experiments provided a clear signal that exposure to 5 to 10 mg/ℓ of BRH material for 14 days negatively affected *M. edulis*. From these data an estimated BRH exposure ≥5 mg/ℓ should be sufficient to adversely affect mussels exposed in the field. The estimated maximum exposure concentration in the field (0.8 mg/ℓ), approximately half that in the lowest laboratory BRH exposure (1.5 mg/ℓ), did not appear to affect the mussels. The mussels were exposed to much higher concentrations of BRH (10×) in the laboratory than in the field. The response in total adenine pool concentrations was elicited only at these higher concentrations and therefore would not be expected in the field.

Nephtys incisa

158. Exposure conditions must be examined to determine whether the biological responses are responding to comparable situations in the laboratory and in the field. Physical data were used to make three different estimates of exposure to BRH material at the FVP stations. Water chemistry data were used to estimate milligrams of BRH per litre 1 m above the bottom at the FVP

stations (Nelson et al. 1987). With the assumption of a 10× enrichment from the 1 m above the bottom value, there is a predicted exposure at the sediment-water interface of 6 to 13 mg BRH/ℓ at the FVP stations as a result of disposal at the FVP site. Estimates of exposure via resuspension of surficial sediments predicted much higher concentrations. A worst case estimate assumes that all of the predicted suspended solids are BRH material from the disposal mound. This estimate predicts up to 100 mg BRH/ℓ under quiescent conditions and up to 300 mg BRH/ℓ under storm conditions. A more probable estimate assumes that sediments resuspended at each station are the source of contaminants for the suspended solids. The estimate predicts a graded exposure at the FVP stations with maximum values of 40 mg/ℓ at 200E, 12 mg/ℓ at 400E, and 4 mg/ℓ at 100E for quiescent conditions. Those values increase to 120 mg/ℓ at 200E, 40 mg/ℓ at 400E, and 10 mg/ℓ at 100E for storm conditions.

159. If it is assumed that tissue concentrations in *N. incisa* are directly related to exposure concentrations, this relationship may be used to test the reasonableness of the exposure predictions. This assumption is reasonable, based on results from laboratory experiments. A cluster analysis of all *N. incisa* tissue residue data revealed no consistent clustering of the laboratory data separate from the field data. Therefore, if it is assumed that tissue concentrations reflect exposure concentrations, then this association of laboratory and field tissue concentration data indicates an overlap of laboratory exposure conditions with field exposure conditions. The estimates of field exposures to BRH sediment (milligrams per litre) suspended at the sediment-water interface based on PCB concentrations in field-collected *N. incisa* are up to 12 mg/ℓ at REFS, 88 mg/ℓ at 100E, and 30 mg/ℓ at 400E.

160. Assuming that exposures were due to initial dispersion of BRH sediments during disposal and subsequent resuspension and movement of sediments from the dredged material mound, a combination of estimates seems appropriate. The estimate based on water chemistry predicts exposures of at least 6 mg/ℓ at the sediment-water interface at all FVP stations during disposal activities in CLIS. The worst case resuspension estimate predicts exposures of up to 100 mg/ℓ in the vicinity of the disposal mound. These estimates (6-100 mg/ℓ) agree well with those predicted by the tissue concentration exposure concentration relationship (12-130 mg/ℓ). The two laboratory exposures for the sister chromatid exchange response were 0 and 200 mg BRH/ℓ as suspended solids. These exposures overlap the estimated range of exposures in the field,

simulated clean control conditions at REFS and worst case storm conditions near the disposal mound.

161. In a prior laboratory study (Zaroogian et al. 1985), *N. incisa* were allowed to burrow into either 100-percent REF or 100-percent BRH sediment prior to treatment with suspended REF or BRH sediments at concentrations up to 200 mg/ℓ. These experiments lasted for 10 days, and no differences in AEC occurred among treatments. Thus, the results of this prior study were in agreement with the present study where no differences in AEC or adenine nucleotide concentrations occurred among treatments. The longest experiment in the present study was 6 weeks.

162. In contrast to the laboratory results where no differences were reported among treatments, field studies showed highly significant differences among stations in September 1983 (T + 16 weeks) for AEC and all adenine nucleotide concentrations. This response in the field coincides with peak exposures as evidenced by highest tissue concentrations of PCBs measured in field *N. incisa*. There is marked difference between laboratory and field responses in spite of estimated comparable exposures to BRH sediments. For example, the highest concentration of PCB measured in tissues of laboratory *N. incisa* was 1,500 ng/g dry weight (sampled at 42 days, 100-percent BRH treatment), while the highest concentration of PCB measured in tissues of field *N. incisa* was 1,250 ng/g dry weight (sampled 6 September 1983 at 400E). A major difference between laboratory and field studies was the duration of exposures: 6 weeks in the laboratory versus 16 weeks in the field. The importance of exposure time suggests that the PAHs may be affecting the adenine nucleotide concentration responses. The residue-effect relationships between tissue concentrations of PAHs and adenine nucleotide concentrations responses in laboratory *N. incisa* further support this suggestion. These compounds may be metabolized to reactive intermediates. A key enzyme system in this metabolism is the cytochrome P-450 dependent mixed-function oxygenase (MFO) system. These enzymes are not functioning at all times. Their reactivity is induced, that is, greatly increased, by exposure to substrate compounds (PAHs). This induction is not immediate. Fries and Lee (1984) report an induction time of 4 to 8 weeks for the MFO system in the marine polychaete *Nereis virens* exposed to benzo(a)pyrene.

163. The contrasting patterns of increased tissue concentrations of PCBs and PAHs in *N. incisa* during the summer of 1983 indicate that the MFO

system in the exposed worms had been activated. PCB tissue concentrations reached a peak in September, 4 months postdisposal, indicating a continuous exposure to contaminated sediments during this period. In contrast to the 4-month period of PCB increase, the highest PAH tissue concentrations occurred in July, only 2 months postdisposal. Laboratory bioaccumulation data suggest that *N. incisa* can metabolize PAHS and that this metabolic capability was induced during the 42-day experimental period. These data suggest that metabolism of PAHs was induced and was causing a sharp decline in tissue PAH concentrations despite continuous exposure to these compounds. Presumably, by 16 weeks postdisposal, the worm MFO system had been induced, resulting in a significant biological response.

164. The results of this study suggest that the activity of the MFO system in *N. incisa* may be an important variable because of the high concentrations of compounds, such as PAHs in BRH sediment, metabolized by these enzymes. The MFO system is induced by exposure to contaminants such as PAHs. Because this induction takes weeks in polychaetes, the laboratory experiments were probably not long enough to induce maximum MFO activity. The shortness of laboratory experiments with *N. incisa* in the FVP program was cited as a limitation also in the cytogenetic studies (Pesch et al. 1987). The simplest explanation of why the laboratory and field results do not match for *N. incisa* in this study is that the laboratory experiments were not long enough. The field data suggest that the laboratory experiments should be conducted for periods of at least 4 months. The results suggest also that MFO activity should be monitored in organisms exposed to material such as BRH sediments.

Residue-Effects Comparisons

165. Bioaccumulation of contaminants depends on various pharmacokinetic processes including uptake, distribution, metabolism, and elimination. The extent to which a contaminant will bioaccumulate depends on the relative rates of uptake versus rates of metabolism and elimination. A contaminant that is metabolized and eliminated rapidly, for example, probably will not bioaccumulate.

166. Bioaccumulation data provide evidence that contaminants are biologically available. This is useful information for complex wastes such as BRH sediments. However, bioaccumulation data alone provide no evidence of an

effect or consequence to the organism. The relevance of a specific tissue residue to the fitness of an organism is unknown.

167. In this study, an attempt was made to identify relationships between tissue concentrations of selected BRH sediment contaminants and biological effects under both laboratory and field exposures. It was assumed that these selected contaminants were biologically available and had toxicological properties that would affect adenine nucleotide concentrations in *N. incisa* and *M. edulis*. The difficulty was that BRH sediment contained many contaminants, any of which alone could be toxic to these test species and possibly more toxic in combination. Thus, it is impossible to determine if the accumulation of any of the selected contaminants was responsible for any observed effect. Therefore, any relationship between body burden and biological response can be attributed only to the sediment, although inferences or trends may be identified with individual contaminants.

168. Adenine nucleotides and AEC are of interest in measuring stress effects because of their central role in energy transformation and their importance as regulators of metabolic processes. Vetter and Hodson (1984) state that the metabolic costs of stress may result from either:

> (1) diverting assimilated energy to nonproductive functions such as increased respiration, cell repair, cleaning and avoidance activity or (2) decreasing the efficiency of energy transfer reactions through toxic effects on enzyme systems, changes in membrane potentials or genetic damage. Either mechanism results in an increase in the dissipation of assimilated energy as heat and a concomitant decrease in growth and reproductive potential.

This study was not designed to distinguish between these mechanisms. Therefore, the adenine nucleotide and AEC responses measured in *M. edulis* and *N. incisa* could be due to either or both mechanisms.

Mytilus edulis

169. The strong exposure-residue relationships measured in the laboratory experiments indicate that the contaminants in BRH sediments are biologically available. Regression analysis was used to determine whether any relationships existed among the five biological measures (AEC, concentrations of ATP, ADP, AMP, and total adenylate nucleotide pools) and tissue concentrations of the selected 10 chemicals and 2 summary statistics for PAHs.

170. In the laboratory, significant correlations existed between total

adenylate pool and sum of 178 alkyl homologs, fluoranthene, benzo(a)pyrene, sum of PAHs, PCB as 1254, ethylan, copper, and cadmium. This represents a broad spectrum of chemicals, including several classes of organic compounds plus metals. It is interesting to note that, in these same experiments, significant correlations existed between scope for growth responses and the exact same list of chemicals, (Nelson et al. 1987). Scope for growth, as with the adenine nucleotide pools, is a measure of energy utilization. Thus, these two independent measures suggest that these chemicals affected energy flow and utilization in exposed *M. edulis*.

171. Detoxification systems for a wide range of chemical compounds are known to exist in marine organisms. An important feature of these systems is that they are inducible; that is, their functional activity is greatly increased by exposure to substrate chemicals. Therefore, organisms having these systems are able to acquire tolerance to and the ability to excrete a variety of chemicals. The primary detoxification systems so far identified are the MFO system for organic xenobiotics and the metallothioneins for metals. These systems apparently are universally distributed, and *M. edulis* has both MFO (Livingstone 1985) and metallothionein (Roesijadi 1982, 1986). These systems function to sequester contaminants and protect against direct toxic action. Theoretically, an organism is protected until the detoxification systems exceed saturation. Only when contaminants "spill over" do they have direct toxic impact on an organism. However, as Jenkins and Brown (1984) point out, this approach "ignores the potential indirect cost of detoxification to an organism. This cost may represent the energy required for the synthesis of detoxification proteins, competition for amino acids or the physical cost of accumulating large quantities of proteins and/or membrane-bound vesicles within a cell."

172. Declines in adenine nucleotide energy pools and scope for growth indices (Nelson et al. 1987) indicate that *M. edulis* was stressed by exposure to BRH sediments in the laboratory experiments. This stress was characterized by increased energy costs. Measurements of the adenine nucleotide concentrations can help to characterize the energy costs incurred by organisms under stressful conditions. "However, the information content of the basic measurements can be vastly increased and the mechanism of toxic action can be suggested if the changes in adenylate concentration are considered in the light of changes in other energy compounds such as glycogen and neutral lipid

reserves," state Vetter and Hodson (1984). Ultimately, energy costs of stress need to be related to changes in growth and reproduction and to impacts on populations.

173. In contrast to the laboratory results, there were no strong exposure-residue relationships measured in the field. Cluster analysis of laboratory and field mussel residue data yielded results similar to those obtained in estimating field exposures. Maximum exposures in the field were half that of the lowest BRH exposure in the laboratory. These exposures existed during disposal of BRH sediments at the FVP site and for several months postdisposal. Thereafter, there was no measurable accumulation of contaminants in *M. edulis* tissues, indicating little or no exposure to BRH sediments in the field during the 2 years of postdisposal monitoring. The contaminant residues in the field mussels were most similar to those of mussels exposed to reference sediment in the laboratory. Thus, the exposure data and the tissue residue data indicate that in the field *M. edulis* was exposed to low and transient levels of BRH sediments. The effects data support this hypothesis.

174. Use of adenine nucleotides as indicators of stress presumes that the three adenylates remain almost constant within an organism except under conditions of extreme energy consumption or of metabolic inhibition. When the concentrations of the three adenylates change in response to stress, these changes occur in consistent and predictable ways. This concept suggested that the ratios of adenylate concentrations, as represented by AEC, were a useful measure of an organism's well-being. As a response to stress, it is expected that ATP concentration will decrease, AMP and ADP concentrations will increase, and, therefore, AEC will decrease. The residue-effect data for *M. edulis* from the field are inconsistent with these expectations. If increased tissue concentrations of contaminants are to be construed as stressful, then concentrations of ATP should be negatively correlated, concentrations of ADP and AMP should be positively correlated, and AEC should be negatively correlated. The field data for *M. edulis* show an opposite pattern. Concentration of ATP was positively correlated with tissue concentrations of PCB. Concentrations of ADP and AMP were negatively correlated with tissue concentrations of five contaminants. AEC values were positively correlated with tissue concentrations of five contaminants. The apparent AEC response to iron further confirms the inconsistent nature of these data. Iron is present

in all sediments in relatively high concentrations. It is not specific to BRH sediments. Also, it is relatively nontoxic. It was included in the list of 12 chemical variables as a negative control. That is, detection of any biological response to iron concentrations was not expected. The adenine nucleotide concentrations and AEC values indicate that FVP field-collected *M. edulis* may have been responding to something, but that something was not tissue concentrations of contaminants, nor was it exposure to BRH sediments.

Nephtys incisa

175. The strong exposure-residue relationships measured in the laboratory experiments indicate that the contaminants in BRH sediments are biologically available. Regression analysis was used to determine whether any relationships existed between the five biological measures (AEC, concentrations of ATP, ADP, AMP, and adenine nucleotide pools) and the tissue concentrations of the selected 10 chemicals and 2 summary statistics for PAHs.

176. Significant correlations were detected between the following responses and chemical contaminants: the adenine nucleotides correlated with PAHs, phenanthrene, sum of 178 homologs, benzo(a)pyrene, and the sum of the PAHs; measures of ATP correlated with PAHs, phenanthrene, benzo(a)pyrene, and the sum of the PAHs; ADP showed similar correlations as ATP but with the addition of PCBs; finally, AEC correlated with phenanthrene and benzo(a)pyrene. This represents a narrow spectrum of chemicals; 12 of 13 significant correlations were with PAHs. In contrast, the physiological measure of net growth efficiency responded to a broad spectrum of chemicals, including several classes of organic compounds plus metals in these same laboratory experiments (Johns and Gutjahr-Gobell 1988). The data suggest that the adenine nucleotide pools in *N. incisa* were affected by the PAHs in BRH sediment.

177. Ironically, a direct correlation between tissue concentrations of PAHs and a biological response is not always expected. For high molecular weight PAHs, it is not the parent PAH compounds that are biologically damaging. Damaging action is associated with metabolic products. These metabolites inflict damage when the affected organisms are actively depurating. Therefore, a simple correlation between tissue concentration of parent PAH compounds and biological response will not exist generally.

178. PAHs are metabolized by the MFO system. Polychaetes are known to have active, inducible MFO systems (Lee et al. 1981; Lee and Singer 1980). Induction of these enzymes takes weeks to months (Fries and Lee 1984). It is

during maximum activity of the MFO system that maximum biological effect would be expected. It is instructive on this point to compare laboratory and field residue-effects data for the adenine nucleotide responses. This comparison is complicated somewhat by technical difficulties encountered during the early field measurements (Zaroogian et al. 1985). However, with this constraint, some general observations may be useful. In the field there were no meaningful significant correlations. The single exception, iron, was included as a negative control. There were no correlations between tissue concentrations of PAHs and any measure of adenine nucleotide response. Yet, at 16 weeks post-disposal, there were highly significant differences in adenine nucleotide responses among field stations. In the laboratory, there were significant correlations between tissue concentrations of PAHs and adenine nucleotide responses. Yet, in the laboratory, there were no significant differences in adenine nucleotide responses among treatments. Exposure data indicate comparable exposures in the laboratory and in the field. The big difference between laboratory and field was length of exposure. The maximum length of laboratory exposure was 6 weeks. The maximum response in the field was at 16 weeks. The data suggest that, in the laboratory at 6 weeks, depuration had been initiated, residue-effect correlations had been established, but no significant biological responses had been evoked. In the field, at 16 weeks, depuration had actively depleted most of the tissue concentrations of PAHs, residue-effects correlations were no longer present, and significantly different biological responses among stations were present as a consequence. If this interpretation of the data is accurate, it suggests that the laboratory experiments were not long enough. More time was needed to induce fully the MFO system in *N. incisa*. The induction of the MFO system is crucial to an organism's response to complex materials, such as BRH sediments, that contain high concentrations of PAHs.

179. Overall, it appears that a number of factors other than BRH sediments were affecting the AEC response in the field. Even where statistically significant responses were observed, they were indicative of nonstressed organisms and probably have little, if any, ecological significance.

PART V: CONCLUSIONS

180. There were three primary objectives in the FVP. The first objective was to test the applicability of biological responses to measure effects of dredged material. The second objective was to field verify responses observed in the laboratory and determine the accuracy of the laboratory predictions. The third objective was to determine the degree of correlation between tissue residues accumulated from dredged material and biological responses as observed in both the laboratory and the field.

181. The biological responses evaluated in this report included the adenine nucleotide measures of ATP, ADP, AMP, adenine nucleotide pool, and AEC. These responses were measured in *M. edulis* and *N. incisa* exposed to BRH sediment in the laboratory and in the field. The only significant laboratory response was a reduction in total adenylate pool concentration measured in *M. edulis* at BRH exposure concentrations higher than any estimated exposures in the field. The only significant field responses were station-related changes in all adenylate nucleotide concentrations measured in *N. incisa* 16 weeks postdisposal. This represented an exposure lasting 10 weeks longer than the longest laboratory exposure, which was 6 weeks. The field exposures were indicative of nonstressed organisms and appeared not to result from exposure to BRH sediments.

182. The adenine nucleotide pool concentrations in organisms exposed to BRH sediments will respond in a concentration-related manner. However, these responses are relatively insensitive in *M. edulis* and are related to long exposure periods in *N. incisa*.

183. The apparent differences between laboratory and field responses for *M. edulis* and for *N. incisa* could be explained by differences in exposures between the two situations. For *M. edulis*, the exposures were much higher in the laboratory. For *N. incisa*, the exposures in the laboratory and the field were comparable, but the field response was significant at 16 weeks postdisposal. This length of exposure far exceeded the length of any laboratory experiments.

184. Adenine nucleotide and AEC are important in energy transformation and in regulation of metabolic processes. Therefore, it is not surprising that responses in adenine nucleotide pools correlate with tissue concentrations of BRH contaminants in exposed organisms. Measurements of the adenine

nucleotide concentrations may help to characterize the energy costs incurred by organisms under stressful conditions.

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APPENDIX A: BLACK ROCK HARBOR SEDIMENT PERCENTAGE CALCULATIONS

Table A1

Percentage of Black Rock Harbor (BRH) Sediment in the Surficial Sediments (0-2 cm) and the Contaminants Used for the Percent Calculations

Date	Station			
	CNTR	200E	400E	1000E
	Percentage of BRH Sediment			
Jun 83	44.5	41.1	12.5	1.8
Jul 83	15.0	37.4	3.3	1.6
Sep 83	32.0	36.7	4.9	2.0
Dec 83	32.8	36.1	9.5	4.4
Mar 84	4.4	2.2	1.9	1.8
Jun 84	9.5	15.6	0.5	0.7
Sep 84	10.0	0.8	3.5	0.5
Oct 84	2.6	--	0.2	1.6
Dec 84	35.1	11.3	0.0	1.0
Oct 85	0.2	21.0	0.0	0.0

(Continued)

Table A1 (Concluded)

Date	Station			
	CNTR	200E	400E	1000E
		Contaminants Used*		
Jun 83	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cd+Cu+Cr	Cd+Cu+Cr
Jul 83	PAH+Cd+Cu+Cr	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cd+Cu+Cr
Sep 83	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cd+Cu+Cr
Dec 83	Cd+Cu+Cr	Cd+Cu+Cr	Cd+Cu+Cr	Cd+Cu+Cr
Mar 84	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cu+Cr
Jun 84	Cd+Cu+Cr	Cd+Cu+Cr	Cd+Cu+Cr	Cu+Cr
Sep 84	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cd+Cu+Cr	PAH+PCB+Cu+Cr	PAH+PCB+Cu+Cr
Oct 84	PAH+PCB	--	PAH+PCB	PAH+PCB
Dec 84	Cd+Cu+Cr	Cd+Cu+Cr	Cu+Cr	Cu+Cr
Oct 85	PCB	PAH+PCB	PCB	PAH+PCB

* PAH = polynuclear aromatic hydrocarbons; PCB = polychlorinated biphenyls.

Table A2

Phenanthrene Concentrations (ng/g Dry Weight) in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
8/18/82	--	--	--	--	--
11/11/82	--	--	--	--	--
12/8/82	--	--	--	--	114
12/8/82	--	--	--	--	77
3/2/83	105	101	132	--	107
3/2/83	--	--	--	--	98
3/2/83	--	--	--	--	62
6/3/83	1,560	1,960	910	52	88
6/3/83	--	--	--	63	--
7/26/83	770	1,710	240	174	51
9/1/83	780	1,010	220	168	94
9/1/83	--	--	--	--	81
3/19/84	77	98	100	250	42
3/20/84	--	--	141	78	90
3/20/84	--	--	--	--	76
3/20/84	200	--	--	--	--
9/11/84	147	57	116	109	40
10/16/84	230	--	85	137	123
10/22/85	43	440	38	69	51

Table A3

178 Alkyl Homolog Concentrations (ng/g Dry Weight) in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
8/18/82	--	--	--	--	--
11/11/82	--	--	--	--	--
12/8/82	--	--	--	--	210
12/8/82	--	--	--	--	172
3/2/83	250	210	260	--	188
3/2/83	--	--	--	--	230
3/2/83	--	--	--	--	127
6/3/83	--	--	5,300	230	189
6/3/83	--	--	--	122	--
7/26/83	9,700	--	1,500	412	131
9/1/83	5,200	--	1,480	613	186
9/1/83	--	--	--	--	189
3/19/84	1,330	590	560	600	103
3/20/84	--	--	590	260	170
3/20/84	--	--	--	--	185
3/20/84	1,200	--	--	--	--
9/11/84	3,000	270	640	250	103
10/16/84	1,260	--	240	420	240
10/22/85	490	3,800	430	210	192

Table A4

Fluoranthene Concentrations (ng/g Dry Weight) in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
8/18/82	--	--	--	--	--
11/11/82	--	--	--	--	--
12/8/82	--	--	--	--	280
12/8/82	--	--	--	--	200
3/2/83	300	260	340	--	270
3/2/83	--	--	--	--	230
3/2/83	--	--	--	--	148
6/3/83	2,300	2,300	1,240	142	220
6/3/83	--	--	--	161	--
7/26/83	1,940	2,600	570	400	140
9/1/83	1,370	2,800	560	380	220
9/1/83	--	--	--	--	210
3/19/84	290	330	330	600	124
3/20/84	--	--	360	210	230
3/20/84	--	--	--	--	185
3/20/84	510	--	--	--	--
9/11/84	650	166	410	250	108
10/16/84	580	--	240	320	300
10/22/85	172	1,770	142	196	189

Table A5

Benzo(a)pyrene Concentrations (ng/g Dry Weight) in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
8/18/82	--	--	--	--	--
11/11/82	--	--	--	--	--
12/8/82	--	--	--	--	280
12/8/82	--	--	--	--	220
3/2/83	260	270	310	--	220
3/2/83	--	--	--	--	210
3/2/83	--	--	--	--	173
6/3/83	1,640	1,490	810	122	210
6/3/83	--	--	--	158	--
7/26/83	1,520	1,750	380	370	169
9/1/83	1,000	2,100	570	320	200
9/1/83	--	--	--	--	230
3/19/84	220	350	260	450	155
3/20/84	--	--	400	280	240
3/20/84	--	--	--	--	185
3/20/84	460	--	--	--	--
9/11/84	600	230	400	260	111
10/16/84	450	--	240	320	290
10/22/85	280	1,130	230	196	380

Table A6

SUM PAH* Concentrations (ng/g Dry Weight) in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
8/18/82	--	--	--	--	--
11/11/82	--	--	--	--	--
12/8/82	--	--	--	--	5,200
12/8/82	--	--	--	--	4,500
3/2/83	5,100	4,900	5,900	--	4,400
3/2/83	--	--	--	--	4,300
3/2/83	--	--	--	--	3,300
6/3/83	62,000	59,000	30,000	2,400	3,900
6/3/83	--	--	--	3,000	--
7/26/83	54,000	63,000	10,100	7,200	3,200
9/1/83	33,000	71,000	13,500	7,200	3,600
9/1/83	--	--	--	--	4,300
3/19/84	7,200	7,100	6,200	9,300	2,700
3/20/84	--	--	7,300	4,500	3,600
3/20/84	--	--	--	--	4,300
3/20/84	11,100	--	--	--	--
9/11/84	18,600	4,400	8,600	5,000	2,000
10/16/84	11,500	--	4,800	6,700	5,800
10/22/85	5,400	34,000	4,900	3,800	5,400

* PAH = polynuclear aromatic hydrocarbons.

Table A7

Centroid Statistic in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
8/18/82	--	--	--	--	--
11/11/82	--	--	--	--	--
12/8/82	--	--	--	--	249.7
12/8/82	--	--	--	--	252.0
3/2/83	247.6	248.9	247.7	--	247.4
3/2/83	--	--	--	--	248.0
3/2/83	--	--	--	--	252.1
6/3/83	238.7	234.1	235.2	241.4	248.3
6/3/83	--	--	--	250.3	--
7/26/83	234.7	232.6	234.4	247.3	252.5
9/1/83	239.7	238.6	244.7	244.3	245.4
9/1/83	--	--	--	--	250.3
3/19/84	237.0	245.1	241.1	244.5	251.0
3/20/84	--	--	243.5	245.3	243.7
3/20/84	--	--	--	--	251.5
3/20/84	242.9	--	--	--	--
9/11/84	240.8	249.2	244.1	247.5	247.2
10/16/84	240.4	--	248.4	247.7	250.0
10/22/85	248.8	241.1	248.6	248.7	253.4

Table A8

Ethylan Concentrations (ng/g Dry Weight)

in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
8/18/82	--	--	--	--	--
11/11/82	--	--	--	--	--
12/8/82	--	--	--	--	0.0
12/8/82	--	--	--	--	0.0
3/2/83	0.0	0.0	0.0	--	0.0
3/2/83	--	--	--	--	0.0
3/2/83	--	--	--	--	0.0
6/3/83	340.0	370.0	163.0	5.0	0.0
6/3/83	--	--	--	0.0	--
7/26/83	0.0	950.0	90.0	35.0	0.0
9/1/83	210.0	670.0	30.0	15.0	0.0
9/1/83	--	--	--	--	0.0
3/19/84	74.0	50.0	36.0	31.0	0.0
3/20/84	--	--	12.0	0.0	0.0
3/20/84	--	--	--	--	0.0
3/20/84	23.0	--	--	--	--
9/11/84	96.0	14.0	64.0	3.0	0.0
10/16/84	12.0	--	2.0	7.0	0.0
10/22/85	8.0	820.0	4.0	5.0	0.0

Table A9

PCB* (A1254) Concentrations (ng/g Dry Weight)

in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
8/18/82	--	--	73	--	59
11/11/82	--	--	30	--	26
12/8/82	--	--	--	--	48
3/2/83	77	75	98	--	65
3/2/83	--	--	--	--	67
3/2/83	--	--	--	--	60
6/3/83	1,730	1,650	890	79	59
6/3/83	--	--	--	45	--
7/26/83	180	1,830	240	117	28
9/1/83	1,190	2,200	340	200	59
3/19/84	270	250	162	96	26
3/20/84	181	--	--	--	--
9/11/84	440	113	183	66	27
10/16/84	181	--	84	162	77
10/22/85	72	1,550	37	48	29

* PCB = polychlorinated biphenyls.

Table A10

Cadmium Concentrations (ng/g Dry Weight)

in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
3/4/83	0.36	0.34	1.06	0.29	0.24
3/4/83	0.39	0.35	0.44	0.21	0.22
3/4/83	0.35	0.49	0.32	0.25	0.22
6/3/83	17.00	13.90	7.30	0.74	0.22
6/3/83	12.40	14.70	4.20	0.58	0.21
6/3/83	13.00	12.90	3.70	0.64	0.19
7/26/83	5.40	11.70	1.14	0.64	0.22
9/1/83	4.10	9.80	0.84	0.68	0.18
9/1/83	21.00	8.70	3.60	0.76	--
12/9/83	8.80	8.70	3.30	1.02	--
3/19/84	2.10	1.11	0.85	1.08	0.20
3/19/84	--	0.87	--	--	--
3/19/84	--	0.23	--	--	--
6/12/84	3.10	4.80	0.37	0.39	--
9/11/84	3.70	0.73	0.97	0.30	0.20
12/20/84	9.30	2.50	0.32	0.72	--
10/22/85	0.45	8.30	0.29	0.32	0.16

Table A11

Chromium Concentrations (ng/g Dry Weight)

in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
3/4/83	56	39	59	59	48
3/4/83	53	57	43	58	52
3/4/83	45	56	56	60	54
6/3/83	870	680	340	69	49
6/3/83	780	740	191	72	48
6/3/83	800	600	155	74	48
7/26/83	120	519	69	66	44
9/1/83	310	600	106	79	56
9/1/83	680	380	160	79	--
12/9/83	520	660	117	126	--
3/19/84	100	52	54	86	47
3/19/84	--	140	--	--	--
3/19/84	--	40	--	--	--
6/12/84	138	210	41	52	--
9/11/84	153	41	128	55	44
12/20/84	550	175	47	88	--
10/22/85	54	430	57	59	40

Table A12

Copper Concentrations (ng/g Dry Weight)

in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
3/4/83	67	57	67	70	55
3/4/83	62	69	63	68	57
3/4/83	63	67	64	69	58
6/3/83	1,640	1,380	680	99	48
6/3/83	1,300	1,420	360	102	51
6/3/83	1,330	1,240	303	106	56
7/26/83	450	1,230	185	106	49
9/1/83	560	1,070	134	103	47
9/1/83	1,890	910	510	122	--
12/9/83	910	950	370	177	--
3/19/84	200	111	143	123	53
3/19/84	--	107	--	--	--
3/19/84	--	114	--	--	--
6/12/84	350	530	89	83	--
9/11/84	430	86	156	73	48
12/20/84	1,000	500	52	131	--
10/22/85	92	910	75	72	46

Table A13

Iron Concentrations (ng/g Dry Weight)

in Surficial Sediments

Date	Station				
	CNTR	200E	400E	1000E	REFS
3/4/83	21,000	17,100	22,000	23,000	19,700
3/4/83	20,000	22,000	18,900	23,000	21,000
3/4/83	18,400	21,000	21,000	23,000	22,000
6/3/83	17,100	19,200	23,000	21,000	21,000
6/3/83	19,300	19,000	22,000	21,000	19,000
6/3/83	17,900	18,700	23,000	22,000	21,000
7/26/83	15,200	16,700	21,000	16,800	21,000
9/1/83	15,100	19,300	21,000	18,400	19,700
9/1/83	26,000	15,100	--	16,400	--
12/9/83	16,500	21,000	19,600	17,500	--
3/19/84	5,800	17,300	20,000	18,700	21,000
3/19/84	--	16,600	--	--	--
3/19/84	--	15,600	--	--	--
6/12/84	6,500	17,100	19,800	15,600	--
9/11/84	12,600	17,400	18,400	18,200	21,000
12/20/84	18,100	17,300	17,400	18,000	--
10/22/85	9,900	17,200	18,100	18,900	17,000

APPENDIX B: CHEMICAL FORMULAS AND FIELD WORM RESIDUE CONCENTRATIONS

Table B1

Chemical Contaminants Selected for Measurement in Both Field and Laboratory Studies

Chlorinated hydrocarbon pesticides

Polychlorinated biphenyls
Ethylan

Aromatic hydrocarbons ≥ molecular weight 166:

Compound Class	Molecular Weight
Fluorene	166
C-1* Fluorene	180
C-2* Fluorene	194
C-3* Fluorene	208
C-4* Fluorene	222
Phenanthrene	178
Anthracene	178
C-1* Phenanthrene/anthracene	192
C-2* Phenanthrene/anthracene	206
C-3* Phenanthrene/anthracene	220
C-4* Phenanthrene/anthracene	234
Fluoranthene	202
Pyrene	202
C-1* Fluoranthene/pyrene	216
C-2* Fluoranthene/pyrene	230
C-3* Fluoranthene/pyrene	244
C-4* Fluoranthene/pyrene	258
Benzanthracene/chrysene**	228
C-1* Benzanthracene/chrysene**	242
C-2* Benzanthracene/chrysene**	256
C-3* Benzanthracene/chrysene**	270
C-4* Benzanthracene/chrysene**	284

(Continued)

* C-1, C-2, C-3, and C-4 refer to the number of methyl groups substituted somewhere in the parent compound.

** These names are representative of the class of polynuclear aromatic hydrocarbons (PAHs) measured at each molecular weight.

Table B1 (Concluded)

Compound Class	Molecular Weight
Benzofluoranthenes	252
Benzo(e)pyrene	252
Benzo(a)pyrene	252
Perylene	252
C-1* Benzopyrene/perylene**	266
C-2* Benzopyrene/perylene**	280
C-3* Benzopyrene/perylene**	294
C-4* Benzopyrene/perylene**	308
Benzoperylene**	276
Dibenzanthracene**	278
Coronene	300
Dibenzocrysene**	302
Hetrocyclic aromatic compounds	
Dibenzothiopehen	184
C-1* Dibenzothiophene	198
C-2* Dibenzothiophene	212
C-3* Dibenzothiophene	226
C-4* Dibenzothiophene	240
Metals	
Cadmium	
Copper	
Chromium	
Iron	
Lead	
Manganese	
Nickel	
Zinc	

* C-1, C-2, C-3, and C-4 refer to the number of methyl groups substituted somewhere in the parent compound.

** These names are representative of the class of polynuclear aromatic hydrocarbons (PAHs) measured at each molecular weight.

Table B2

Complete Formulae for Calculating all SUM and CENT Variables

```
PSUM  = POS166 + POS178 + POS202 + POS228 + POS252 + POS276 + POS278 +
        POS300 + POS302

HSUM  = H1C166 + H2C166 + H3C166 + H4C166 + H1C178 + H2C178 + H3C178 +
        H4C178 + H1C202 + H2C202 + H3C202 + H4C202 + H1C228 + H2C228 +
        H3C228 + H4C228 + H1C252 + H2C252 + H3C252 + H4C252

SUM   = POS166 + H1C166 + H2C166 + H3C166 + H4C166 + POS178 + H1C178 +
        H2C178 + H3C178 + H4C178 + POS202 + H1C202 + H2C202 + H3C202 +
        H4C202 + POS228 + H1C228 + H2C228 + H3C228 + H4C228 + POS252 +
        H1C252 + H2C252 + H3C252 + H4C252 + POS276 + POS278 + POS300 +
        POS302

PCENT = [POS166*166 + POS178*178 + POS202*202 + POS228*228 + POS252*252 +
        POS276*276 + POS278*278 + POS300*300 + POS302*302]/PSUM

HCENT = [H1C166*180 + H2C166*194 + H3C166*208 + H4C166*222 + H1C178*192 +
        H2C178*206 + H3C178*220 + H4C178*234 + H1C202*216 + H2C202*230 +
        H3C202*244 + H4C202*258 + H1C228*242 + H2C228*256 + H3C228*270 +
        H4C228*284 + H1C252*266 + H2C252*280 + H3C252*294 + H4C252*308]/HSUM

CENT  = [POS166*166 + H1C166*180 + H2C166*194 + H3C166*208 + H4C166*222 +
        POS178*178 + H1C178*192 + H2C178*206 + H3C178*220 + H4C178*234 +
        POS202*202 + H1C202*216 + H2C202*230 + H3C202*244 + H4C202*258 +
        POS228*228 + H1C228*242 + H2C228*256 + H3C228*270 + H4C228*284 +
        POS252*252 + H1C252*266 + H2C252*280 + H3C252*294 + H4C252*308 +
        POS276*276 + POS278*278 + POS300*300 + POS302*302]/SUM
```

The sum of alkyl homologs of PAH molecular weight 178 (HOS178) is calculated according to the following formula:

HOS178 = H1C178 + H2C178 + H3C178 + H4C178

where

HOS178 = sum of C-1 to C-4 alkyl-substituted 178 PAHs

This statistic was chosen to describe the alkyl homologs because the 178 alkyl homologs are the most intense homologs within the Black Rock Harbor (BRH) PAH distribution and because they afford the greatest BRH to REFS concentration ratio. Alkyl homologs were included because of potential differences between them and parent PAHs.

Table B3

Tissue Residue Concentrations in Mussels from the T - 4 Field Collection in CLIS (22 Apr 83)*

Chemical Compound	Station			
	CNTR	400E	1000E	REFS
Phenanthrene	210	117	98	38
Sum of 178 alkyl homologs	580	310	310	290
Fluoranthene	161	102	90	82
Benzo(a)pyrene	37	20	34	25
Ethylan	5	3	5	10
PCB as A1254	380	270	400	440
SUM of PAHs	2,600	1,520	1,650	1,380
CENTROID of PAHs	218	219	225	228
Copper	13.5	15.1	14.5	12.5
Cadmium	1.9	1.8	1.8	1.8
Chromium	1.8	3.8	2.2	1.6
Iron	370	1,400	530	340

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic CENTROID.

Table B4

Tissue Residue Concentrations in Mussels from the T + 0 Field Collection in CLIS (24 May 83)*

Chemical Compound	Station** 1000E	REFS
Phenanthrene	43	16
Sum of 178 alkyl homologs	1,440	290
Fluoranthene	161	52
Benzo(a)pyrene	100	18
Ethylan	102	9
PCB as A1254	1,080	500
SUM of PAHs	5,400	1,290
CENTROID of PAHs	230	232
Copper	16.5	10.9
Cadmium	2.0	2.3
Chromium	2.6	1.5
Iron	420	330

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic CENTROID.

** The CNTR station was not deployed because of the dumping operation and the 400E station was lost.

Table B5

Tissue Residue Concentrations in Mussels from the T + 2 Field Collection in CLIS (07 Jun 83)*

Chemical Compound	Station** 400E	1000E	REFS
Phenanthrene	69	41	13
Sum of 178 alkyl homologs	1,900	970	540
Fluoranthene	290	126	72
Benzo(a)pyrene	210	118	51
Ethylan	71	39	17
PCB as A1254	1,440	1,020	630
SUM of PAHs	8,700	4,700	2,500
CENTROID of PAHs	232	234	233
Copper	16.9	15.6	10.8
Cadmium	2.3	2.3	1.9
Chromium	3.0	3.0	2.0
Iron	510	560	560

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic CENTROID.

** The CNTR station was not deployed because of the disposal operation.

Table B6

Tissue Residue Concentrations in Mussels from the T + 8 Field Collection in CLIS (10 Jul 83)*

Chemical Compound	Station			
	CNTR	400E	1000E	REFS
Phenanthrene	11	14	9	7
Sum of 178 alkyl homologs	350	340	193	105
Fluoranthene	45	46	31	23
Benzo(a)pyrene	40	50	18	20
Ethylan	22	20	7	1
PCB as A1254	700	740	620	480
SUM of PAHs	1,870	2,100	1,020	760
CENTROID of PAHs	234	236	231	240
Copper	10.1	9.6	11.5	4.4
Cadmium	1.9	2.0	1.3	0.9
Chromium	1.4	1.4	3.2	0.8
Iron	340	370	820	240

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic CENTROID.

Table B7

Tissue Residue Concentrations in Mussels from the T + 12 Field Collection in CLIS (10 Aug 83)*

Chemical Compound	Station			
	CNTR	400E	1000E	REFS
Phenanthrene	17	10	9	8
Sum of 178 alkyl homologs	250	160	96	65
Fluoranthene	41	28	20	15
Benzo(a)pyrene	41	17	16	13
Ethylan	9	8	3	1
PCB as A1254	640	660	550	570
SUM of PAHs	1,600	940	710	530
CENTROID of PAHs	237	236	239	240
Copper	5.3	5.6	7.5	5.8
Cadmium	0.9	0.9	1.2	1.1
Chromium	1.0	0.7	1.6	0.7
Iron	164	167	450	177

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic CENTROID.

Table B8

Tissue Residue Concentrations in Mussels from the T + 15 Field Collection in CLIS (06 Sep 83)*

Chemical Compound	Station			
	CNTR	400E	1000E	REFS
Phenanthrene	13	9	10	6
Sum of 178 alkyl homologs	370	230	210	43
Fluoranthene	57	38	33	14
Benzo(a)pyrene	53	45	28	7
Ethylan	10	6	4	1
PCB as A1254	870	630	640	550
SUM of PAHs	2,100	1,540	1,240	350
CENTROID of PAHs	236	239	237	238
Copper	7.7	6.0	8.0	5.8
Cadmium	1.0	1.1	1.1	0.9
Chromium	1.2	0.9	1.1	0.9
Iron	260	179	290	260

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic CENTROID.

Table B9

Tissue Residue Concentrations in Mussels from the T + 21 Field Collection in CLIS (18 Oct 83)*

Chemical Compound	Station			
	CNTR	400E	1000E	REFS
Phenanthrene	12	11	11	10
Sum of 178 alkyl homologs	132	101	88	46
Fluoranthene	33	25	22	16
Benzo(a)pyrene	24	9	17	9
Ethylan	2	2	1	0
PCB as A1254	540	680	570	420
SUM of PAHs	1,000	670	700	400
CENTROID of PAHs	240	234	239	238
Copper	22.1	16.3	15.1	16.4
Cadmium	5.1	4.4	4.8	5.0
Chromium	2.3	2.6	2.2	2.2
Iron	440	540	420	480

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic CENTROID.

Table B10

Tissue Residue Concentrations in Mussels from the T + 27 Field Collection in CLIS (29 Nov 83)*

Chemical Compound	Station** 400E	1000E	REFS
Phenanthrene	18	10	8
Sum of 178 alkyl homologs	230	117	86
Fluoranthene	68	36	37
Benzo(a)pyrene	39	32	19
Ethylan	3	1	0
PCB as A1254	540	380	450
SUM of PAHs	1,820	1,150	860
CENTROID of PAHs	240	244	240
Copper	16.4	21.0	23.3
Cadmium	3.2	3.4	3.6
Chromium	2.5	3.6	3.3
Iron	570	920	920

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic CENTROID.

** The CNTR station was missing at the time of collection.

Table B11

Tissue Residue Concentrations in Mussels from the T + 43 Field Collection in CLIS (20 Mar 84)*

Chemical Compound	Station** CNTR	400E	REFS
Phenanthrene	6	18	17
Sum of 178 alkyl homologs	94	78	70
Fluoranthene	28	26	24
Benzo(a)pyrene	8	4	7
Ethylan	2	1	1
PCB as A1254	350	330	280
SUM of PAHs	510	460	450
CENTROID of PAHs	229	230	231

* Metals were not measured for these samples. Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, and molecular weight for the statistic CENTROID.

** The 1000E station was missing at the time of collection.

Table B12

Tissue Residue Concentrations in Mussels from the T + 55 Field Collection in CLIS (05 Jun 84)*

Chemical Compound	Station** 400E	1000E	REFS
Phenanthrene	6	6	4
Sum of 178 alkyl homologs	89	91	54
Fluoranthene	25	31	18
Benzo(a)pyrene	6	7	6
Ethylan	0	1	0
PCB as A1254	540	490	550
SUM of PAHs	520	550	370
CENTROID of PAHs	234	235	236

* Metals were not measured for these samples. Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, and molecular weight for the statistic CENTROID.

** The CNTR station was missing at the time of collection.

Table B13

Tissue Residue Concentrations in Mussels from the T + 116 Field Collection in CLIS (13 Aug 85)*

Chemical Compound	Station			
	CNTR	400E	1000E	REFS
Phenanthrene	3	6	3	3
Sum of 178 alkyl homologs	79	124	80	58
Fluoranthene	18	27	24	19
Benzo(a)pyrene	18	40	22	17
Ethylan	1	2	1	0
PCB as A1254	310	350	450	440
SUM of PAHs	700	1,270	810	620
CENTROID of PAHs	242	243	241	244
Copper	8.5	7.5	6.9	7.6
Cadmium	1.5	1.3	1.3	1.3
Chromium	1.2	1.1	0.9	0.9
Iron	290	260	220	220

* Units are nanograms per gram dry weight for the organic compounds and the statistic SUM, micrograms per gram dry weight for the inorganic elements, and molecular weight for the statistic CENTROID.